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CONTENTS

OKADA, YÔ K. *Supplementary Note on the Double Formation in the Echinus Germ in Diluted Sea Water* 75

DAWSON, J. A., AND BELKIN, MORRIS *The Digestion of Oils by Amœba proteus* 80

BUTCHER, EARL O. *The Origin of the Germ Cells in the Lake Lamprey (Petromyzon marinus unicolor)* 87

FRY, HENRY J. *The So-called Central Bodies in Fertilized Echinarachnius Eggs* 101

NABOURS, ROBERT K., AND FOSTER, MARTHA E. *Parthenogenesis and the Inheritance of Color Patterns in the Grouse Locust Paratettix texanus Hancock* 129

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BIOLOGICAL BULLETIN

SUPPLEMENTARY NOTE ON THE DOUBLE FORMATION IN THE *ECHINUS* GERM IN DILUTED SEA WATER.

YÔ K. OKADA

NABA, HYOGO-KEN (JAPAN).

"The eggs of sea-urchins absorb so much water in the diluted sea water that their membranes burst and part of their protoplasm flows out. The eggs then consist of two connected spheres of protoplasm, as the extruded part of protoplasm in consequence of its surface tension assumes a spheric form, as does the protoplasm remaining behind inside the membrane." On treating the eggs of *Arbacia* 10 minutes after fertilization with sea water, to which 100 per cent. of its volume of distilled water had been added, and on replacing them in ordinary sea water, J. Loeb (1894) had the good fortune to obtain double or triple larvæ developed from the egg with extra-ovates. The experiments were repeated by B. Rawitz (1896) at Rovigno with the eggs of *Strongylocentrotus*, by F. A. Janssens (1904) at Naples with the eggs of *Arbacia*, and by N. Yatsu (1910) with those of Neapolitan *Echinus*, *Arbacia* and *Strongylocentrotus*. All obtained extra ovates but did not succeed in getting multiple embryos.

Doubt has been cast upon this part of Loeb's account of the artificial production of twin embryos, but in 1926 G. Fadda not only confirmed the double gastrulation in the eggs with extra-ovates, but theoretically demonstrated the causes of its occurrence. ' Wenn die Blastomeren nur einer einzigen Kugel angehören, wird die Differenzierung eine einheitliche sein, weil alle andere nachfolgenden Blastomeren an der ersten dicht anliegen können. Wenn aber eine Einschürung von einem gegebenen Grade gewisse Blastomeren verhindert, von einer Krümmung zur anderen überzugehen, dann werden zwei Punkte der Differ-

enzierung entstehen, durch die Einschnürungsfurche getrennt, sodass auf diese Weise die Bildung einfecher oder doppelter Embryonen von der Tiefe dieser Furche und mithin vom Durchmesser der — — — — beiden abgeschnürten Teile abhängt." Applying the formula of Giglio-Tos—

$$\delta = \sqrt{\frac{r^2 + r'^2}{2}}$$

so far as δ remains larger than half of the square root in the sum of squares of the diameters of both parts, namely that of the egg (r) and that of the extra-ovate (r'), the embryonic formation will be simple, while when δ becomes smaller than the value in question, the formation will be double. δ may be the same value as the right-hand side of the equation, and then the formation cannot be determined by this method; it is sometimes simple, sometimes double as the case may be.

Coming back to Yatsu's observations, the eggs with extra-ovates, though they failed to develop into multiple embryos, are still capable of dividing and "some in *Arbacia*, and all in *Strongylocentrotus*, cleaved more or less abnormally." Under the same treatment the fertilization membrane in the eggs of *Echinus* does not burst, and there is consequently no extra-ovation. "This is due" states Yatsu, "to the fact that in *Echinus* the space between the egg and the membrane is so wide that the turgid egg does not reach the membrane while in a hypotomic solution."

According to M. Konopacki (1918) such eggs of *Strongylocentrotus lividus* "können sich bis zum Larvenstadium in einer aus 70 Teilen Seewasser und 30 Teilen Süsswasser bestehenden Lösungen entwickeln" but "in einer Lösung 60/40 können sie kaum drei bis vier Furchungen durchlaufen."

The eggs of the Japanese *Strongylocentrotus* (*S. plucherimus*) show still stronger resistance to the dilution of sea water with distilled water than the European form does, and I have got plutei (if not entirely normal ones) even in a mixture of 14 parts of sea water and 6 parts of distilled water, *i.e.*, about 37 per cent. of distilled water against only 23 per cent. in Konopacki's experiment.

Up to a dilution of 10 per cent. of distilled water, the eggs of *Strongylocentrotus pluclierimus* develop normally and the plutei formed do not differ from those grown in the normal medium. Plutei developed in a solution of 14 parts of sea water and 6 parts of distilled water are without arms, but the structure of their alimentary tract and body skeleton is quite normal. Unexpectedly, only in those lots in 16 parts of sea water plus 4 parts of distilled water there appears an interesting monstrosity. In three of my experimental glasses, more than one third of the larvæ all have the same abnormality, the posterior part of their body being widened, and more or less bifid. Such a larva has generally double sets of the body rods in its skeletal framework, while in the anterior part the ciliary bands, the arms, as well as the alimentary tract, remain normal as in the ordinary pluteus of the species. A representation of the larvæ is shown in Fig. 1*a*, which is sketched from the living, with a camera lucida.

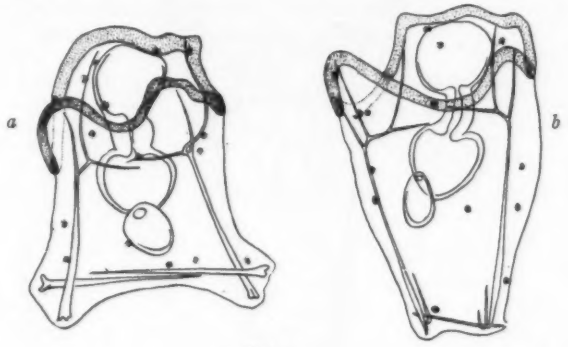

FIG. 1.

Besides the monsters just mentioned there were in another glass, larvæ in which the abnormality was not so pronounced as before. One of these is shown in Fig. 1*b*. In this latter type, the posterior end of the body is neither so broad nor so distinctly bifid as in the first type, and there is only one extra spicule, which is transversely placed between two body rods at the posterior end of the body; the body rods in the normal larva approach closely to one another and one may even fall upon the other.

Comparing these two types of the abnormal plutei, it is easy

to suppose that the second type is a less modified condition of the first. The duplication of the plutei in this case is due to the appearance of an extra transverse bar of spicules connected with the process of widening the posterior part of the body. The single extra bar then divides into two, and each gives rise to an independent, extra body rod. It remains now for us to consider whether the extra transverse bar of spicules always appears when the posterior end of the pluteus widens or whether it is a special effect of the dilution of sea water with distilled water.

We can easily produce plutei, with a broader posterior extremity, by treating fertilized eggs of *Strongylocentrotus pulcherimus* for a certain time (before the blastula stage), with a weak Li-Cl solution, and then replacing them in normal sea water. Fig. 2 represents one such production. The posterior

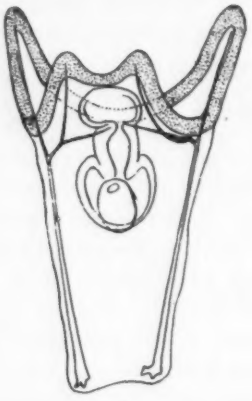

FIG. 2.

end of the pluteus is unmistakably widened, even more than in the second type of the preceding experiment, but there is no appearance of the extra transverse bar of spicules this time, nor, of course, of the extra body rods, which now may be considered specific to the dilution of sea water with distilled water.

A fairly large number of different types of double monsters have already been described in echinoid larvæ, but I do not know of another case of such partial bifurcation of the posterior

part of the body, with double sets of skeletons, and with the anterior part and the alimentary tract remaining entirely normal. Hence the reason for this small note, which also demonstrates that double formation is possible in the eggs of sea urchins, which have developed in diluted sea water, even when extra-ovates have not been produced.

REFERENCES

Fadda, G.

'26 Über die Bildung der Doppelembryonen. Arch. f. Entw'mech. Vol. 107, p. 213.

Janssens, F. A.

'04 Production artificielle des larves geantes et monstreuses dans l'*Arbacia*. La Cellule, Vol. 21.

Konopocki, u.

'18 Untersuchungen über die Einwirkung verdünten See-wassers auf verschiedene Entwicklungs-Stadien der Echinoiden (*Strongylocentrotus lividus*). Arch. f. Entw'mech., Vol. 44, p. 337.

Loeb, J.

'94 Über eine einfache Methode Zwei oder mehr zusammenwachsene Embryonen aus einem Ei hervorzubringen. Pfluger's Arch., Vol. 55, p. 525.

Rawitz, B.

'96 Über den Einflux verdünnter See-wasser auf die Furchungsfahigkeit der Seeigel Eier. Arch. f. Anat. u. Phys. (Phys. Abth.).

Yatsu, N.

'10 Extra-ovate Experiments on the Egg of Sea-urchin. Ann. Zool. Jap., Vol. F, p. 213.

THE DIGESTION OF OILS BY *AMŒBA PROTEUS*.

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In a previous paper by the writers ('28) an account was given of the digestion of various oils by *Amœba dubia*. The present account deals with a series of similar experiments performed under similar conditions in which the same oils were used with another species of large free-living ameba, *Amœba proteus* (Schaeffer, '16). The procedure in this work was in every respect identical with that used with *Amœba dubia*, except that none of the oils used in this series were radiated. In the previous account as well as in this no attempt to study the rate of break-down of the various oils has been made; the sole object has been to establish the fact that a considerable variety of oils has been definitely acted on by the amebae. A study of the rate and nature of the break-down process is now in progress.

The result of this series of injections may be tabulated as follows:

TABLE I.

Oil.	No. of Amebæ Injected.	No. of Amebæ Digesting Oil.	Ave. Total Vol. in μ^3 Digested per Ameba.	Ave. No. Days Injected Amebæ Lived.	Ave. No. Days Control Amebæ Lived.
1. Codliver.....	17	7	27,600	7–8	7–8
2. Cottonseed..	20	7	22,300	7.5	7.5
3. Olive.....	21	13	13,000	10.9	10.1
4. Peanut.....	21	17	12,600	8.5	6.6
5. Sperm.....	23	10	9,600	8.0	8.0
6. Linseed.....	15	8	8,800	7.5	9.0
7. Oleic.....	28	9	6,400	6.4	6.0
8. Oxfoot.....	13	8	5,300	6.0	5.4
9. Nujol.....	17	0	0,	6.0	6.0

The outstanding fact in this series of experiments with *A. proteus* is the same as in the former series with *A. dubia*, *i.e.*, that different oils are broken down. As can be seen by a comparison of the figures in the tables the relative digestibility of the

oils appears to differ in *A. proteus* and *A. dubia*. We wish, however, to point out that no great significance should be attached to these figures as such. They should be considered only in a broad relative aspect, although our observations lead us to the belief that distinctive differences do exist.

In any comparison of the relative volumes digested the respective volumes of the amebae must be considered. The volume of *A. proteus* was determined by the same method as that used for *A. dubia*. The average of measurements of 50 representative amebae gave an average volume of approximately 1,000,000μ^3.[1] If the ability of amebæ of these two species to break down oils is quantitatively similar it should be expected that the amounts of oil digested would be in the same ratio. The results indicate that *A. dubia* breaks down a greater volume of oil on the average than *A. proteus* although from the data given there is slight adherence to this ratio.

In the case of oleic acid and linseed oil (non-radiated in the *A. proteus* series as shown in Table 1 some digestion took place whereas none occurred in *A. dubia*. With nujol, an inert paraffin oil, as in the previous work no break-down was expected or found.

As can be seen from Table 1 there is no significant difference in most cases between the length of life of controls and experimental animals. In the case of olive and peanut oils the experimental animals lived longer on the average than the controls. In the case of linseed oil, non-radiated, the controls lived longer than the experimental animals. This effect with linseed oil may be due to the presence of traces of lead and other heavy metals which analysis showed to be present in the oil.

The entire process of breaking down of the oils in *A. proteus* is, so far as observation shows, entirely similar to the phenomenon in *A. dubia*. As in *A. dubia*, digestion did not take place in every case of injection (See Table 1). The reasons for the variation in digestion of any one oil among the individual amebæ are no doubt many; but important factors are the physiological condition of the ameba as a whole and its immediate condition from a nutritive standpoint. Temperature conditions were the same for all cases. The temperature, however, was not controlled; all the experiments being done at room temperature which varied between 68° and 74° F.

So far as our observations and data warrant it thus appears that oils of diverse types are successfully broken down by both *A. proteus* and *A. dubia* in a similar manner. Further attempts are now in progress to ascertain the precise physiological nature of this reaction in these two species of amebæ.

Some Significant Differences between *A. dubia* and *A. proteus*.

The two species of large free-living amebæ which have been used by the writers in a preceding paper ('28) and in the present work were first adequately described by Schaeffer ('16). In a recent publication (Dawson, '28) mention has been made of the fact that in long-continued mass cultures the specific differences as pointed out by Schaeffer are retained. During the course of the micro-injection studies carried on by the authors a number of fundamental differences between these two species have been disclosed.

1. *Differences in the Ability to Break Down Oils.*

As has been pointed out above the two species of amebæ show some differences in their reactions to injected oils. *A. dubia* not only did not break down the oleic acid, oxfoot and linseed oils used in this series but retained these oils for relatively short periods after injection. *A. proteus* on the other hand, in numerous cases retained and broke down these same oils.

2. *Morphological Differences.*

From the very beginning of our work difficulties in manipulative technique when working with *A. proteus* indicated that the nature of the pellicle (outermost layer) differed from that of *A. dubia*. When attempting to inject *A. proteus* using a fairly fine pipette with a slender shaft (about 2–3 μ in diameter) bending of the pipette could be seen to take place, whereas in injection of *A. dubia* such bending rarely or never occurred. If a pipette of larger diameter (about 7–8 μ) was used the pellicle of *A. proteus* in direct contact with the pipette could invariably be seen to yield or give before the pipette until the two outermost layers of the ameba almost touched each other. Such a pipette could be used to

inject *A. dubia* with comparative ease, and with a minimum amount of yielding of the pellicle before the pipette as the latter was inserted into the ameba. This would seem to indicate clearly that the pellicle of *A. proteus* is tougher than that of *A. dubia.* Our measurements have shown us that *A. dubia* is thicker in cross section than *A. proteus* (70 μ vs. 40 μ). We have found that *A. proteus* does not lend itself as easily to microinjection technique as A. dubia. The greater thinness of *A. proteus* and the toughness of its pellicle may account largely for this difficulty.

3. *Capping.*

Early in the work of injecting oil in *A. dubia* an interesting phenomenon was encountered. In many unsuccessful attempts at injection it appeared that the oil, instead of being injected into the organism, was merely brought into intimate contact with the outermost surface of the ameba. When the pipette was withdrawn the oil did not become dislodged from the surface of the ameba as might be expected but continued to remain attached to it, usually assuming roughly the form of a slightly concave hemisphere with the concave face in contact with the ameba. Whenever this occurred there was instant and typical response on the part of the organism. The protoplasm in contact with the oil extruded to form a pseudopodium, with the oil giving superficially the appearance of adhering to it at its outermost tip, and the main body of the ameba continued to flow into this pseudopodium. In about 30 seconds the entire ameba had assumed the form of the type known as *A. limax* and its endoplasmic streaming and nature of locomotion were in practically every respect identical with or markedly similar to that of an ameba of the "limax" type. In every case the ameba progressed so that the oil was pushed in advance, never dragged behind. This phenomenon the writers have termed "capping." The 'cap' might remain attached for days to the ameba and there was little or no change in either its shape or that of the modified "limax" shape of the ameba. Eventually either the cap was dislodged or the ameba died with the oil still adhering. In no case was there ever noticed any action upon the oil by the ameba which might be observed by appearance of oil in the ameba or by decrease in the size of the cap. This "capping" is

fairly easy to accomplish with *A. dubia* and when once begun invariably goes on to completion. Within one second the cap is formed and the ameba begins to assume the "limax" form immediately. With *A. proteus*, however, in no instance has a cap ever been successfully completed to remain lodged for more than a few minutes on the anterior end of the ameba as is the case in *A. dubia*. In the case of *A. proteus* only the first beginnings of the process take place; the oil when in contact with the ameba results in the formation of a very small pseudopod which protrudes in a manner very similar to that of *A. dubia* but which in a few seconds or, at most, minutes loses its contact with the oil and flows back into the main body of the ameba.

It was found that these caps as described in the preceding paragraph could be formed very frequently if a droplet of oil was forced out of the pipette but permitted to remain attached to it and then approximated to the ameba until it just came in contact and was allowed to remain thus for several seconds. If the oil was then very slightly retracted by withdrawing the pipette the capping process frequently went on to completion doing so with great speed (less than one second). For best results in obtaining caps the diameter of the droplet should not be more than approximately 50 μ. An optimum size is from 25 to 30 μ. If the droplet exceeded 50 μ in diameter there was only slight cap formation followed almost immediately by the breaking away or the separation of the oil from the ameba.

4. *Ingestion.*

During the course of these attempts it was found that if the oil was permitted to remain in contact with the ameba without retraction for several seconds, the ameba reacted by flowing around and over the oil forming a normal food cup, and, when the pipette was gently withdrawn, complete ingestion of the oil took place. After ingestion the oil was moved about in the endoplasm behaving precisely like a droplet which had been injected and was subsequently broken down in exactly the same manner as after injection. It was found that for ingestion to take place the oil had to be supported against the pipette, for if the droplet was ejected into the medium directly in the path of the ameba the

latter extruded pseudopodia toward it but ingestion failed because the streaming pseudopodium pushed the oil away from it. That this difficulty was purely a mechanical one was proved by piercing the same droplet with the pipette and holding it firmly whereupon it was ingested by the ameba. Ingestion did not take place every time a droplet of suitable size was presented to an ameba. Sometimes the ingestion reaction would be repeated several times by the same ameba unsuccessfully, followed by successful ingestion on the presentation of the oil immediately following. As is well known the previous condition of the experimental protozoon in respect to nutrition controls in large part the response in regard to further food taking. Whether this is the case in respect to this phenomenon we are unable to state at present.

The optimum size of oil droplet for ingestion comprises all sizes up to 50 μ. Very small droplets may be ingested but present technical difficulties, as they tend to adhere to the pipette and to be withdrawn from the ameba.

Contrasted with this reaction of *A. dubia* ingestion has never been accomplished with *A. proteus*. With this ameba there is only a very slight attempt at foodcup formation. *A. proteus* always moves away from the oil even when the droplet is brought into contact with the animal and held so for several seconds.

The breakdown of ingested droplets in *A. dubia* takes place so far as observation and quantitative data show in precisely the same way as with injected droplets. So far, ingestion experiments have been carried on with five oils, viz., nujol, olive, codliver, sperm and cottonseed. *A. dubia* ingested all of these oils in the same way. In no case of ingestion of oil droplets was a vacuole ever formed about the oil. Likewise no vacuole was ever formed about any injected oil drop.

5. *Comparative Length of Life of Controls.*

The behavior of these two species of ameba when placed in distilled water as controls was markedly different from the standpoint of length of survival. *A. dubia* lived on the average from 4 to 5 days under these conditions at room temperature. None survived beyond six days. The average length of life for *A. proteus* under the same conditions was from 7 to 8 days with cer-

tain series living up to 11 days, and with numerous instances of individuals living for 18 days. This indicates a greater degree of hardiness on the part of *A. proteus* which is fully substantiated by experience in the mass culture of both these organisms.

Summary.

1. *A. proteus* successfully breaks down, after micro-injection, the following oils: codliver, cottonseed, olive, peanut, sperm, linseed, oleic, oxfoot.

2. A number of significant differences have been found between *A. dubia* and *A. proteus.*

a. In their respective ability to break down oils.

b. Morphological differences as revealed by injection, measurement of volume and nature of pellicle.

c. Under certain conditions *A. dubia* undergoes the phenomenon of 'capping' with oil. No permanent "capping" ever takes place with *A. proteus.*

d. A dubia will under suitable conditions ingest oil. *A. proteus* under similar conditions never ingests oil.

e. A. proteus has the ability to live longer under similar adverse conditions than *A. dubia.*

LITERATURE CITED.

Dawson, J. A.

'28 The Culture of Large Free-living Amebæ. Amer. Nat., Vol. 62, 453–466.

Dawson, J. A., and Morris Belkin.

'28 The Digestion of Oils by *Amœba dubia.* Proc. Soc. Exp. Biol. and Med., Vol. 25, 790–793.

Schaeffer, A. A.

'16 Notes on the Specific and Other Characters of *Amœba proteus* Pallas (Leidy), *A. discoides* spec. nov., and *A. dubia* spec. nov. Arch. f. Protistenk., Bd. 37, 204–228.

[1] Due to error the volume of *A. dubia* was given as 500,000 μ^3. This should be 2,500,000 μ^3. Thus the percentages given in Table 1, column 4, in our previous paper should be multiplied by 0.2.

THE ORIGIN OF THE GERM CELLS IN THE LAKE LAMPREY (*PETROMYZON MARINUS UNICOLOR*).

EARL O. BUTCHER

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INTRODUCTION.

Among the numerous articles concerning the sex cells in the vertebrates, one of the most striking features is the varied opinion on their origin and on the time of their earliest appearance in the embryo. For a long time, this has provided the basis for the much debated question of whether reproductive cells are segregated early or arise from epithelial cells as the result of cellular differentiation.

In lower vertebrates, so-called germ cells are described in the entoderm and mesoderm by various authors (Woods, '02; Wheeler, '99; Humphrey, '25, and others) both before and after the formation of the somites. These cells, which are described as reaching the genital anlage through various means, are usually larger than their immediate neighbors, and have a more distinct nucleus and a fairly definite boundary. The above characteristics and the fact that they can be distinguished from other surrounding cells at an early period lead some (Beard, '02; Allen, '09; Okkelberg, '21, and others) to think that they are early segregated cells. They have not, however, been traced, in most instances, into early cleavage stages, and thus no positive proof of an early separation and a definite germ tract has been offered.

Some of the other lower vertebrates provide well known instances of the formation of germ cells from the peritoneal epithelium. Bouin ('00) in his critical study of amphibia concludes that the reproductive cells have a mesenchymal and a peritoneal origin. In *Rana temporaria,* Gatenby ('16) describes the transition of peritoneal cells into germ cells. In the same manner, Lubosch ('03) accounts for the origin of the sex cells in *Petromyzon planeri.*

Literature provides us with practically no evidence of the presence of germ cells before the appearance of the gonad in higher vertebrates, especially in mammals. Their source here is said to be the peritoneal epithelium and in many cases the first generation of sex cells (Kingery, '17; Winiwarter and Sainmont, '08) is found to degenerate, the definitive cells being formed from the germinal epithelium (Allen, '23; Papanicolaou, '25, and others).

Wherever germ cells are found to be formed from epithelial cells it seems to support the increasing evidence in favor of the theory that sex cells may be differentiated from body cells and do not have a specific character.

It is with one of the cyclostomes, *Petromyzon marinus unicolor*, that the present investigation is principally concerned. The study [1] is made as an effort to ascertain whether germ cells are segregated early, or arise as the result of cellular differentiation.

Observations.

Large cells with round vesicular nuclei, which apparently are identical with those germ cells found by Okkelberg ('21) in the brook lamprey, have been observed by the writer in the caudal end of the embryo just before the appearance of the distinct entoderm and mesoderm. At this time the greater number of these cells lie immediately below the ectoderm ventro-lateral to the position of the future pronephric duct. Their distinct cell boundaries and characteristic roundness render them distinguishable from the irregular surrounding cells. Previous to this time, they are apparently similar in structure to other embryonic cells. As development proceeds and the mesoderm separates from the entoderm, these yolk laden cells are found in the mesoderm. Goette ('90) recognized similar cells (*Petromyzon fluviatilis*) occurring in the mesodermal plates directly ventrad and laterad of the pronephric duct. Large round masses of yolk, distinctly marked off from other entodermal cells just laterad to the myotomes, are considered by Wheeler in Petromyzon planeri to be reproductive cells. He finds that they become included in the mesoderm upon its lateral extension.

[1] The writer desires to express his appreciation for the helpful suggestions of Prof. H. D. Reed. To Prof. Gage he is, likewise, indebted for the loan of a complete series of early lake lampreys.

As the embryo of the lake lamprey becomes older, the number of these yolk-laden cells increases in the mesoderm as it separates from the entoderm caudally. Judging from the series at my disposal, there is apparently no particular arrangement of these cells at first, but with further differentiation of the mesoderm, they become grouped in bands, ventro-lateral to the pronephric ducts. Their characteristic large size and roundness make them easily recognizable among the neighboring cells.

At approximately the time that the larva breaks out of the egg membrane, the cells of the anterior levels reach a more medial position probably through a dorso-medial shifting of structures. In their new location they are now situated, for the most part, ventral to the pronephric ducts. Their roundness of form has been lost to some extent and they now possess a more flattened appearance, due, probably to the pressure of adjacent parts. In the caudal end of the embryo the bands of cells are still in the more lateral position and are widely separated.

In the cephalic region of a larva, older than the one described above, these large groups of cells are seen to practically come together medially. Often the nucleus with its vesicular appearance is hard to distinguish on account of the yolk-laden character of the cells. In the caudal direction, the large cells are still in a ventro-lateral position with respect to the pronephric duct. They retain a round form, in most instances, due to the more embryonic character of this part of the larva.

When the coelomic cavity forms, these yolk-laden cells become included in the somatic mesoderm. Their yolk granules, however, having begun to disappear, remain in a somewhat fragmented condition. As the yolk is gradually absorbed, the cell becomes smaller. By referring to Fig. 1, it will be seen that the chromatin granules in the large spherical nucleus appear large and stain deeply. One and sometimes two nucleoli are present. In the latter instance, one is usually larger and more intensely staining than the other. These large yolk-retaining cells, as Okkelberg claims in the brook lamprey, are always beneath the peritoneal epithelium from the time that it can be recognized as such.

At the same time that these yolk granules are being absorbed and are becoming less distinct, certain of the peritoneal cells

ventral to the aorta and medial to the pronephric ducts begin to enlarge in situ. At this time the cells of the epithelium vary in shape, but most of them are of a flat elongated type. The first indication that some of these possess different potentialities is their increased size and a transition to a more spherical form. From this stage, they undergo a period of fairly rapid differentiation and acquire characteristics of germ cells found later in the definitive gonad.

The formation of germ cells from the peritoneal epithelium in the lamprey is not an entirely new observation. Lubosch ('03) believed that the peritoneal epithelium was the source of germ cells and follicle cells in *Petromyzon planeri*. He, however, did not give a detailed description of the process. On the other hand, since the germ cells were never seen in the peritoneal epithelium and because of their early history and structural characters in *Entosphenus wilderi*, Okkelberg concluded that the sex cells were not derivatives of the epithelium.

The process of the transition of peritoneal cells into germ cells can easily be found in larvæ which are 7–10 mm. in length. In these individuals, numerous cells of the peritoneal epithelium with deep staining nuclei are seen to be gradually changing from an oval to a spherical form (Fig. 3). The cell boundaries begin to appear more distinct. The cytoplasm is clearer as a whole, and seems to be proportionally larger in amount with respect to the nucleus. This is due to growth and also partly, no doubt, to a change in form.

As differentiation proceeds, the nucleus of the transforming cell becomes more vesicular and the chromatin is seen to clump, so to speak, at the intersections of the linen network (Fig. 2). Often two nucleoli are readily distinguishable. Figs. 4 and 7 show that during this time a change is also occurring in the cytoplasm. Besides increasing in amount, although slightly granular, it is becoming somewhat clearer.

In the course of further development, there is a corresponding change in the form of the immediately adjacent cells. They become flattened against the sex cells, presenting the appearance of crescent-shaped structures. From this stage they soon begin a growth up and over, so as to push the reproductive cells inward

(Fig. 6). Fig. 7 shows that some cells reach a greater size than others before being completely covered over by the growth of the adjacent peritoneal cells. In some cases such a large growth is attained that large bulgings are noticeable (Fig. 5).

As the cell is pushed under the epithelium, it enlarges slightly. The final result is a germ cell with moderately well defined cell boundary and a slightly granular cytoplasm. A definite nuclear membrane is noticeable. Oftentimes the reticulum is poorly defined and the deeply staining chromatin granules present a somewhat isolated appearance. A small lightly staining nucleolus and a larger more distinct one are usually present. In comparison to the final stage of the yolk-laden cells, they are identical in structure, and cannot be told from them. Both have reached a position immediately below the peritoneal epithelium.

Successive transitional stages provide evidence that germ cells arise from the peritoneal epithelium. Further support is furnished from the observation that germ cells attain a considerable size in the epithelium of which they are a part before being pushed inward.

This proliferation and formation of reproductive cells from the peritoneal epithelium is noticeable for a considerable time (7–15 mm. larvæ). The ability of the epithelium to transform into germ cells is gradually reduced, however, as the time is approached when a distinct gonad is recognizable.

Only a few enlarging peritoneal cells are found in 16 mm. larvæ. In some places, however, large germ cells are still found as a part of the epithelium. At this stage a thickening, indicating the region of the future gonad, is beginning to appear immediately below the dorsal aorta. The gonad is noticeable as a distinct structure in 18 mm. larvæ. This agrees with the conditions found in *Petromyzon planeri* by Lubosch. The germ cells found continuous with the epithelium at this time are not different structurally from those found in earlier stages.

The gonad appears as a single ventral median diverticulum in a 20 mm. larva (Fig. 9). For the most part, it is made up of germ cells and an epithelial covering, consisting of flat elongated cells with oval nuclei. Some of the germ cells still lie in the dorsal mesenchyme and have not migrated, or have not been included in

the gonad. Epithelial cells are seen to be proliferated inward to furnish the bulk of the follicle cells. The follicular tissue soon isolates the germ cells, forming small follicles. In some cases, the large sex cells are very near the coelomic cavity, the only separation being the intervening cytoplasm of a flattened follicle cell. The structure of the gonad varies somewhat in its longitudinal extent. In some regions, it consists mainly of follicle and germ cells; at other levels, stroma is its chief constituent.

Later in a 26 mm. larva, after the migration of blood vessels and mesenchyme ventrally into the gonad, the sex gland is less dense than at 20 mm. At about this time, the germ cells begin a period of division and ordinary mitotic figures are occasionally encountered. The reproductive cells are arranged both in nests and as single elements. Where the latter condition occurs, the sex cell, in most cases, remains in a resting stage and has not undergone mitotic division. Nests, which are found less frequently, comprise a varying number of secondary germ elements. Already, in some places, the mass of germ cells is being broken up by the continual ingrowth of follicle cells. The gonad is a narrow diverticulum in some regions, being little wider than a single germ cell. At other levels, no reproductive cells are present, and the sex gland consists of a loose stroma and a cuboidal epithelial covering. For the most part, the majority of the germ cells are in a resting condition, though mitotic stages and completely divided cells are found.

The sex gland, meanwhile, is extending in a cephalic and caudal direction. This leads to the question of whether the germ cells of these extremities arise as a result of division of resting cells or are formed anew from the epithelium of the growth areas. In the larvæ which I have studied, evidence indicates that the former occurs in most instances. Numerous mitoses are found in the peritoneal epithelium but there is little evidence of a great transition of epithelial cells of these regions into reproductive cells.

In 30–33 mm. larvæ, the gonad extends a greater part of the length of the coelom. In Fig. 8 it is also seen to be considerably larger than in the 20 mm. larva of Fig. 9. Some of the germ cells are still in the resting condition, but, apparently, the greater amount of division occurs at this time, from the frequent mitotic

figures encountered. Many nests with various numbers of secondary sex elements are present. The reproductive cells, for the greater part, are peripherally situated in the gonad, being directly under the epithelium. The central part of the sex gland consists mainly of loose stroma and blood vessels. No maturation phenomena are observed in larvæ of this stage.

In the sex gland of a 50 mm. larva, the nests of germ cells are arranged around a medulla of connective tissue which is continuous dorsally with that of the mesentery. Evidently the nest is differentiated as a whole. Some nests contain, for the most part, cells in a resting condition, while in others various mitotic figures are found. In very large nests indications of degeneration are found. Since this appears in only the large ones, the insufficient blood supply and crowding are probably the causes. It is impossible to say whether all the cells of an individual nest result from the mitosis of an original single germ cell, since the migration of follicle tissue inward causes a breaking up and a continual rearrangement. At this time, the sex cells are apparently of an indifferent character, and the sex of the individual could not be established with certainty. Beyond this stage, a detailed study was not made. The maturation stages and the various factors that may be influential in deciding sex were not investigated.

Very few large cells are found to originate from the epithelium after the formation of the gonad. In a 33 mm. larva a few peritoneal cells are found enlarging and figure 8 shows a large reproductive cell continuous with the epithelium of the sex gland. Since the proliferation is usually not great in larvæ as old as this, this is regarded as an unusual occurrence, depending in all probability upon the environmental changes, and various conditions existing within the gonad. Of course, there is the possibility that germ cells might arise from follicle cells or be proliferated inside the gonad before a great growth is attained. In all the material examined, however, there is no indication of such a phenomenon. If there is much formation from the peritoneum after a distinct gonad is recognizable, one would expect to find large and enlarging cells in the epithelium. The increased number of reproductive cells after the appearance of the gonad results, mainly, I believe from mitotic division of germ cells already differentiated.

Discussion.

In this study of the origin of the germ cells, the first question that arises is the one concerning the fate of the yolk-retaining cells, distinguished as primordial germ cells by some authors. Apparently from the previous investigations on vertebrates, they have two alternatives; either they persist to form the definitive germ cells of the adult, or they degenerate and disappear, the definitive sex cells then arising from peritoneal cells. Just how extensive this degeneration is seems to vary in the different vertebrates studied. Bouin holds that most of the primordial germ cells degenerate in *Rana temporaria* and the definitive sex cells have both a mesenchymal and a peritoneal origin. According to Dustin ('07), in Amphibia (*Triton alpestris, Bufo vulgaris* and *Rana fusca*) some of the primordial germ cells which do not degenerate form functional cells in the gonad. He states, however, that a second generation from the epithelial covering of the gonad furnishes the greater portion of the definitive sex cells. Okkelberg believes that the primordial germ cells are the sole source of the definitive cells in the brook lamprey, and none are formed from the epithelium.

In Petromyzon marinus unicolor, most of the large yolk-laden cells, which for the sake of clearness may be termed primordial germ cells, after losing their yolk are indistinguishable from those sex cells having a peritoneal origin. From their position many of them would naturally become included in the gonad. My material shows that some may degenerate, and evidently the rest persist and finally become definitive germ cells. Most of the definitive sex cells, however, probably originate from peritoneal cells. Of course, there is the possibility that these epithelial cells which differentiate into germ cells were the primordial germ elements that reached this position through shifting of structures. That this is not the case, is evidenced by the fact that ordinary epithelial cells indistinguishable from others of the peritoneum are seen to transform into germ cells. It is, likewise, illogical to conceive of the primordial germ cells dedifferentiating into cells indistinguishable from other peritoneal cells and then again gradually take the characteristics of sex cells. To believe that these peritoneal cells are not somatic cells, but cells which have maintained

their embryonic structure and have not specialized in a particular direction would seem even more unreasonable.

If germ cells do not have an epithelial origin, there is no way of accounting for the varying sizes found, since it is highly improbable that the so-called primordial germ cells would exhibit such a variation in size and such a close relation and resemblance to the peritoneal cells. Although large germ cells may appear to be continuous with and actually a part of the epithelium, this does not necessarily justify, in my mind, the conclusion that they have a peritoneal origin. To be certain that germ cells are derived from the epithelium, it is necessary for one to identify successive transitional stages.

Another important question is the significance of the primordial germ cells. Structurally and morphologically at one time, they are indistinguishable from other embryonic cells in the lamprey. Since they cannot be traced back to cleavage stages, there seems to be no logical justification for a belief in a distinct line of germ cells which differ physiologically from other embryonic cells. If, then, there is apparently no basis for the evidence of an early germ tract, the question naturally arises why some cells differ from other embryonic cells in having a yolk-retaining property for so long a time.

Two suggestions are given in the following pages as possible explanations for the presence of these so-called primordial germ cells. In the first place, it is evident that, at one time, the primordial germ cells are in the same position as the cells which later become part of the peritoneal epithelium, but as the latter are organized, the yolk-retaining cells take their position below the peritoneum. If this is the case, the process of germ cell formation is no different from that in later stages (transition of peritoneal epithelial cells into germ cells). The whole thing represents, then, a continuous process of germ cell formation as a result of cellular differentiation from a very early stage up until, and after in some particular cases, the formation of the gonad. If one accepts this analysis, the fundamental property of growth and differentiation becomes the explanation of germ cell formation. No doubt, the reason for degeneration, in some instances, would be the environmental factors, insufficient vascular supply,

and unfavorable conditions existing within the body of the individual.

The second suggestion is one from a phylogenetic point of view. In some arthropods, where all the egg and larval material is laid out for the formation of definite areas in the adult, germ cells may be specially provided with a large amount of nutrient material (Gatenby '24). How this extra supply of food material acts is not known, but as Gatenby intimates it may contain enzymes or other substances which suppress the differentiation of the germ cells during the embryo formation, and keeps them isolated. This, no doubt, prevents their passage into the stream of differentiating somatic cells, whose influence might result in the loss of germ-cell integrity. In mammals, on the other hand, where evidence is increasing that a somatic cell may become a germ cell under the proper stimulus, and where no special provision is made, as in insects, there is found no proof of segregation in cleavage stages. In these higher vertebrates, the cell nucleus has apparently unlimited power and germ cell formation is the result of growth and differentiation.

In the lamprey, it may be that these large yolk-retaining cells are the remnants of the conditions found in lower forms. The germ cells which are formed from peritoneal cells represent a step in that evolutionary process which reaches its height in the mammals.

Even a third interpretation might be presented, namely, that the primordial germ cells may act as a stimulating factor to the formation of germ cells from the epithelium and a gonad. They are found in many vertebrates, yet positive proof that they give rise to all of the definitive sex cells is lacking.

Further comment does not seem necessary. It is evident that the definitive germ cells have, for the most part, a peritoneal origin in the lake lamprey, yet there are primordial germ cells, the presence of which is perplexing.

Conclusions.

1. The definitive sex cells of the lake lamprey originate from the so-called primordial germ cells and also from peritoneal cells.
2. The primordial germ cells are first distinguishable as large

yolk-laden cells in the caudal end of the embryo just before the appearance of a distinct mesoderm and entoderm.

3. The finding of the successive stages from the ordinary epithelial cell to the definitive germ cell offers evidence that germ cells are formed from the peritoneal cells.

LITERATURE CITED.

Allen, B. M.
'09 The Origin of the Sex Cells of *Amia* and *Lepidosteus*. Anat. Rec., Vol. 3.
Allen, Edgar.
'23 Ovogenesis during Sexual Maturity. Am. Jour. Anat., Vol. 31, no. 5.
Beard, J.
'02 The Numerical Law of the Germ Cells. Anat. Anz., Bd. 21.
'02 The Germ Cells in *Pristiurus*. Anat. Anz., Bd. 21.
Bouin, M.
'00 Histogenese de la glande genitale femelle chez *Rana temporaria* (L). Arch. de Biol., T. 17.
Dustin, A. P.
'07 Recherches sur l'origine des gonocytes chez les Amphibiens. Arch. de Biol., T. 23.
Gatenby, J. Bronte.
'16 The Transition of Peritoneal Cells into Germ Cells in Some Amphibia *Anura*, especially in *Rana temporaria*. Quart. Jour. Micr. Sc., Vol. 61.
'24 The Transition of Peritoneal Epithelial Cells into Germ Cells in *Gallus Bankiva*. Quart. Jour. Micr. Sc., Vol. 68.
Goette, A.
'90 Entwicklungsgeschichte des Flussneunauges (*Petromyzon fluviatilis*). Abhandlungen zur Entwicklungsgeschichte der Thiere, Heft. 5, Teil 1. Hamburg u. Leipzig.
Humphrey, R. R.
'25 Primordial Germ Cells of *Hemidactylium* and Other Amphibia. Jour. Morph., Vol. 41, no. 1.
Kingery, H. M.
'17 Oögenesis in the White Mouse. Jour. Morph., Vol. 30, no. 1.
Lubosch, W.
'03 Uber die Geschlectsdifferenzierung bei Ammocoetes. Verb. Anat. Ges. Vers., 17.
Okkelberg, Peter.
'21 The Early History of the Germ Cells in the Brook Lamprey, *Entosphenus wilderi* (Gage), up to and Including the Period of Sex Differentiation. Jour. Morph., Vol. 35, pp. 1–152.
Papanicolaou, George N.
'25 Oögenesis during Sexual Maturity as Elucidated by Experimental Methods. Soc. Exp. Biol. and Med., Vol. 21, p. 393.
Wheeler, W. M.

'99 The Development of the Urogenital Organs of the Lamprey. Zoöl. Jahrb., Abth. Anat., Bd. 13, 1–88.

Winiwarter, H. von, et J. Sainmont.

'08 Nouvelles rechérches sur l'ovogènese et l'organogenese de l'ovaire des Mammifères (chat). Arch. de Biol., T. 24.

Woods, F. A.

'02 Origin and Migration of the Germ Cells in *Acanthias*. Am. Jour. Anat., Vol. 1, pp. 307–320.

EXPLANATION OF FIGURES.

FIG. 1. Transection of a 6.5 mm. larva, showing primordial germ cell in which the yolk is disappearing. × 380.

FIG. 2. Transection of a 9 mm. larva, showing the second stage in the transition of a peritoneal cell into a germ cell. × 640.

FIG. 3. 9 mm. larva, showing the first stage in the enlargement of a peritoneal cell. × 640.

FIG. 4. Enlargement of a definitive sex cell in the peritoneal epithelium of a 7 mm. larva. × 380.

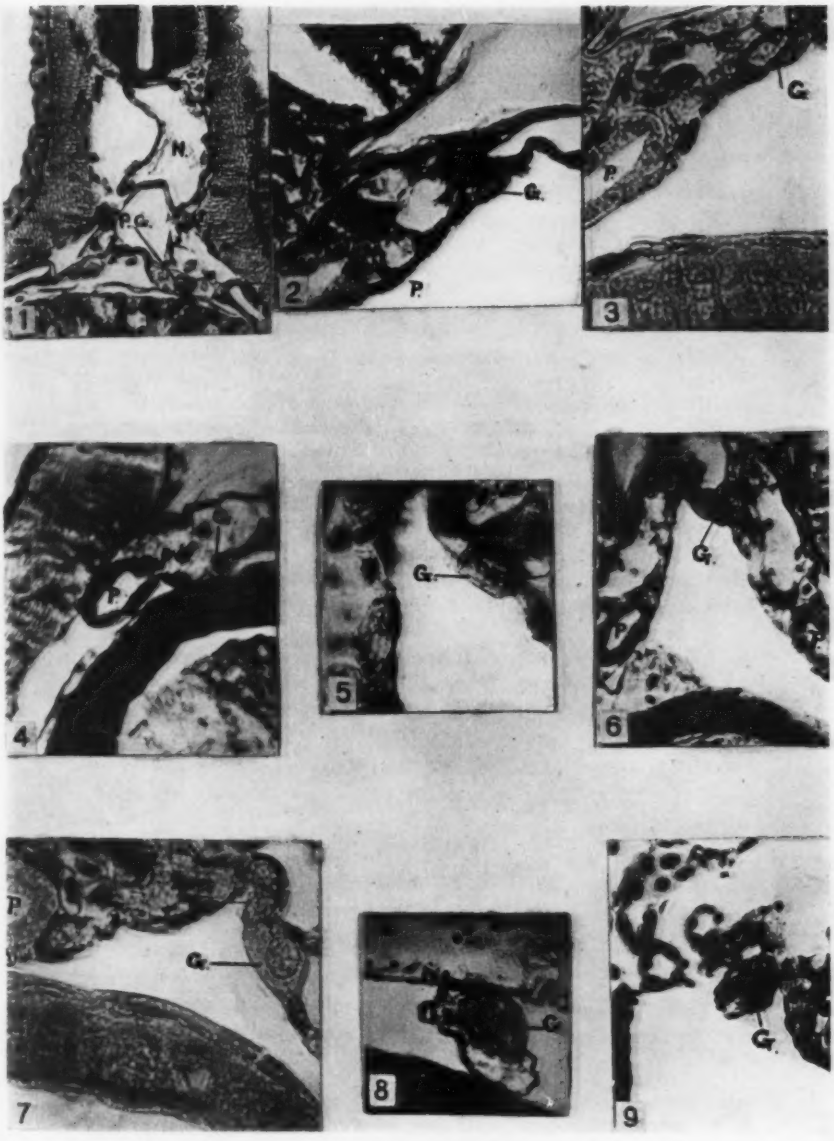
FIG. 5. This illustrates the large size that some germ cells reach before being proliferated. Section of a 10 mm. larva. × 790.

FIG. 6. Section through a 10 mm. larva. The adjacent epithelial cells are growing up around the germ cell. × 380.

FIG. 7. 9 mm. larva. Enlarged germ cell is shown in the epithelium. × 790.

FIG. 8. Gonad of a 33 mm. larva. Germ cell is continuous with the epithelium. × 380.

FIG. 9. Section through gonad of a 20 mm. larva. × 380.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THE SO-CALLED CENTRAL BODIES IN FERTILIZED *ECHINARACHNIUS* EGGS.

I. The Relationship between Central Bodies and Astral Structure as Modified by Various Mitotic Phases.[1]

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Table of Contents.

Introduction.
Hypotheses Concerning the Mitotic Rôle of Central Bodies 101
Morphology and Terminology 104
Previous Studies of Central Bodies in Echinoderm Fertilization ... 105

The Relationship between Central Bodies and Astral Structure as Modified by Various Mitotic Phases in Fertilized *Echinarachnius* Eggs (Bouin's Fixation).
Method of Study 109
History of the Sperm-aster 113
History of the Cleavage-amphiaster 115

Discussion.
The So-called Central Bodies in Fertilized *Echinarachnius* Eggs .. 118
The So-called Central Bodies in Fertilized Echinoderm Eggs 120
Hypotheses Concerning the Mitotic Rôle of Central Bodies in the Light of the Present Investigation 121

Résumé 123

Literature Cited 126

Introduction.

Hypotheses Concerning the Mitotic Rôle of Central Bodies.

Certain hypotheses have been proposed concerning the mitotic rôle of central bodies (centrioles, centrosomes) in the animal cell. There is a diversity of opinion in evaluating these generalizations since the evidence is conflicting and uncertain at many points. An examination of recent cytological textbooks reveals their present status.

[1] The experiments were performed at the Marine Biological Laboratory, Woods Hole, Massachusetts, 1927. A preliminary report of the paper was presented at one of the Research Seminars of the Laboratory, July 31, 1928.

It is generally taken for granted that central bodies are the formative foci about which the mitotic mechanism arises, and that they are its most persistent element. Central bodies "play a preponderant part in the mechanism of cell division" (Doncaster, '20, p. 21); they are the "dynamical centers of the cell" (Agar, '20, p. 4); about them "as a center arise the asters" (Wilson, '28, p. 26).

There is diversity of opinion, however, as to whether or not central bodies maintain genetic continuity from one cell generation to another. On the one hand there is evidence that they arise by the growth and division of preëxisting bodies of the same kind and keep their identity as individualized structures throughout the cell history. Such behavior is shown during the final divisions of the germ cells in many species. On the other hand there are cases where they seem to arise de novo, as in cytasters of artifically activated eggs. The question, therefore, presents itself, do central bodies have a dual mode of origin, in some cases maintaining genetic continuity from cell to cell, but in others arising de novo; or can the two modes be brought under one category? Wilson ('23 and '28) has given most consideration to this subject. "In the very fact of such a double mode of origin (if it can be accepted) lies the peculiar interest of the central bodies in their relation to the protoplasmic metastructure" ('28, p. 672). As a possible explanation of cases where they disappear and later reappear during the cell history, he suggests that ". . . the centrioles are bodies of such extreme minuteness that if not surrounded by astral rays they might readily be lost to view among the protoplasmic granules of the egg or they may even become reduced to ultra-microscopical dimensions" ('28, p. 444). "When, therefore, they seem to make their appearance de novo in the hyaloplasm it is entirely possible that they may preëxist in a form too minute to appear above the horizon of visibility" ('28, p. 720). "Manifestly it is quite illogical to affirm an origin de novo of any formed body because it first becomes visible at a particular enlargement, even the greatest at our present command" ('23, p. 24).

In contrast to this attempt to save the theory of genetic continuity with reference to central bodies that seem to arise de novo,

is the view of other authors such as Sharp. He suggests ('26, p. 188) that " . . . the regular appearance of the centrosome in successive mitoses is closely associated with regularly recurring physiological conditions in the cell; and . . . its presence in successive cell-divisions does not require an uninterrupted morphological continuity through the intervening stages." Thus it is seen that there is diversity of opinion concerning the genetic continuity of central bodies as individualized structures from cell to cell.

There is equally conflicting evidence and opinion with reference to their genetic continuity from generation to generation. The usual hypothesis concerning the rôle of central bodies in fertilization is as follows: " Clearly, . . . something is introduced into the egg by the middle-piece of the sperm that either is a central body or has the power to incite the formation of one; . . . some kind of specific genetic relation exists between the central bodies of successive generations " (Wilson, '28, p. 441). " There is no doubt that the middle-piece of the spermatozoon is at least partly formed from the centrosome of the cell which gives rise to the spermatozoon, and that after fertilization the centrosomes of the first segmentation division of the egg arise from or in connection with the middle-piece " (Doncaster, '20, p. 43). In the fertilized eggs of about five species (Yatsu, '09, pp. 371–372) the central body phenomena seem to be in harmony with this hypothesis, but even in these most favorable cases the evidence is uncertain at various points. Furthermore, there are many species where the theory is without support. Hence a wide range of attitudes is found among different authors toward the hypothesis. Agar ('20, pp. 74–76) accepts it with but slight reservations. On the other hand, Lillie ('19, pp. 70–75), and Lillie and Just ('24, pp. 461–464) practically reject it. Wilson ('28, p. 438–449) gives the fullest account of the facts. He gives greater weight to the hypothesis than do most authors, because of his interest in the theory of genetic continuity as applied to cytoplasmic components. He is very careful to observe, however: " Even in the typical case (e.g., in the sea-urchin, tunicate or nematode) two difficult questions still remain, namely, whether the cleavage-centers are actually derived from the sperm-center, and whether the latter is

actually brought into the egg by the sperm ('28, p. 443). Additional discussions concerning the rôle of central bodies are given by Brachet ('17), and Hertwig ('23).

The present status of the hypotheses concerning the mitotic rôle of central bodies in the animal cell may be summed up as follows. There is general agreement that they are the formative foci about which the astral mechanism arises. There is a conflict of opinion as to whether or not they maintain genetic continuity from cell to cell and from generation to generation; there is a tendency in some quarters to tentatively regard such an assumption as a fruitful working hypothesis.

Morphology and Terminology.

Wilson fully discusses the morphology and terminology of central bodies. The following résumé of the subject is composed of sentences and phrases quoted verbatim from him ('28, pp. 30, 119, 672–680). "Much confusion still exists in the literature concerning the terminology and relationships of the central body. Its most constant and essential component is the *centriole,* a minute granule or rod, often double, in some cases lying naked in the cytoplasm, more often surrounded by a cytoplasmic investment of various degrees of complexity. In some cases the latter is a rather definite small rounded spheroid, the *centrosome;* when larger it is often spoken of as the *sphere* (or *centrosphere*). The word "centrosome" will be found in the literature in at least four different senses, namely: (1) In a general physiological sense as the division center of the cell. (2) As the innermost differentiated body at the center of the aster, the only persistent element of the whole system, equivalent to the central granule or centriole. (3) In Boveri's original sense as a larger body surrounding the centriole, having a persistent identity and independent of the aster. (4) As a transitory structure, representing the innermost astral zone. In practice it is often difficult to distinguish certainly between centriole and centrosome; it was this ambiguity that led Flemming ('91) and later Heidenhain and many others, to adopt the more inclusive and noncommital term central body, (which is historically the older), which leaves open the question as to its precise homology in any particular case. The facts indicate that

the particular type of configuration in the centrosome is a matter of secondary importance, and that the centriole constitutes the most stable and constant feature of the whole astral system of which it forms the center."

The fact that the terms, *central body, centriole,* and *centrosome,* are used in different ways by various authors causes much confusion. It would be desirable if the term *centriole* were applied only to a minute, period-like type of central body, such as occurs in spermatogenesis. The term *centrosome* should designate only the more diffuse types.

Previous Studies of Central Bodies in Echinoderm Fertilization.

The chief studies of central bodies in echinoderm fertilization have been made by Wilson ('95), (Wilson and Leaming '95; Wilson and Mathews '95), and Boveri ('00). Boveri illustrates the early sperm-aster of *Echinus* eggs as centered about a body

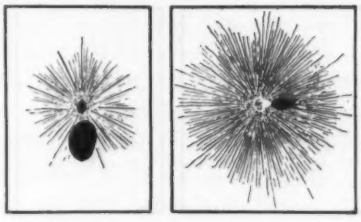


FIG. 1. FIG. 2.

Central bodies in sperm-asters of *Echinus* (Boveri, '00). Figs. 1 and 2 are reproduced from Boveri, '00, Plate V., Fig. 72, and Plate IV., Fig. 55*b*. For discussion see pp. 105 and 120.

containing a granule that may be single or double (Figs. 1 and 2). He interprets these phenomena as a centrosome containing a centriole that divides into two; about this structure arises the sperm-aster; later the two centrioles become the centers of the cleavage-amphiaster. Various workers have clearly established the fact that this configuration is actually a bi-lobed granule of chondriosome material contained within the sperm's middle-piece (Field, '95; Wilson, '97, '99; Meves, '12; and Just, '27). When sectioned in various planes it gives the appearances Boveri illustrates. During the earliest history of the aster this structure is at its

center, but within several minutes it moves to one side of the astral focus, and within about ten minutes it wanders out at random into the cytoplasm, having no further connection with the mitotic mechanism (Figs. 11 to 16). Hence Boveri's observations concerning central bodies of the sperm-aster may be dismissed.

In Wilson's earlier work ('95), (Wilson and Mathews, '95; Wilson and Leaming, '95) the central body of the sperm-aster in *Toxopneustes* is illustrated as a rather large, homogeneously granular structure, which draws apart into two portions during the late astral history. (The middle-piece was mistaken for the central body during the earliest astral stages). At no time is

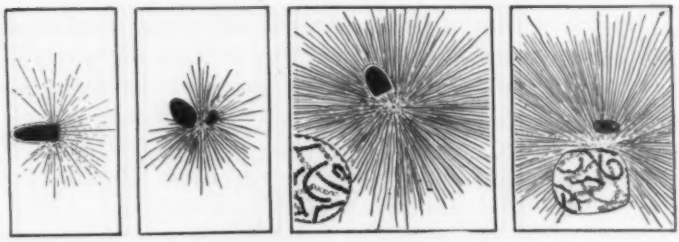


FIG. 3. FIG. 4. FIG. 5. FIG. 6.

Central bodies in sperm-asters of *Toxopneustes* (Wilson, '00). Figs. 3 to 6 are reproduced from Wilson, '00, Figs. 94*E* to 94*G*. For discussion see pp. 106 and 120.

there a minute, period-like centriole. These figures are similar to those of the present study (Figs. 11 to 18). They have been reproduced in text-books only occasionally, e.g., Doncaster ('20, Plate 12). In the first edition of "The Cell" ('96, p. 138) Wilson reproduces the same illustrations but in the discussion he points out that modifications of the original interpretation are necessary (cf. also Wilson, '97 and '99). In the second edition of "The Cell" ('00, p. 189) these modifications are embodied in a new series of illustrations (Figs. 3 to 6). The aster is at first focused about the middle-piece (Fig. 3); the latter soon moves to one side and the focus is occupied by a typical period-like centriole (Fig. 4); somewhat later the middle-piece disappears from the astral area but the centriole is still present (Fig. 5); at later stages the centriole is not shown (Fig. 6). At no time is there the large,

diffuse type of central body illustrated in the earlier work. The later figures, showing a typical minute centriole, have been widely copied not only as delineating the events of echinoderm fertilization specifically but also as showing the typical phenomena of fertilization generally (Hertwig, '23, Fig. 250; Lillie, '19, Fig. 7; Lillie and Just, '24, Fig. 7; Wilson, '28, Fig. 184; Buchner, '15, Fig. 110; etc.).

The following types of central bodies in echinoderm sperm-asters are illustrated in other studies. A large, vaguely-delimited, homogeneously-granular body without a period-like centriole (a configuration similar to that shown by Wilson ('95 and '96) in his earlier work on *Toxopneustes*) is illustrated by Wilson and Mathews ('95) in *Asterias* and in *Arbacia;* and by Ziegler ('04) in *Echinus.* A period-like centriole, without a surrounding homogeneously-granular body (a configuration similar to that shown by Wilson ('00) in his later work on *Toxopneustes*) is illustrated by Kostanecki ('96) in *Echinus;* by Wilson ('23, Fig. 12) in *Asterias;* and by Wilson ('01) in *Toxopneustes* (etherized). A period-like centriole (or centrioles) within a surrounding homogeneously-granular body is illustrated by Hill ('95) and by Erlanger ('98) in *Sphærechinus.*

Turning from observations concerning central bodies in the sperm-aster to those of the cleavage-amphiaster, it is found that Wilson ('95, '96, '00, '28) illustrates structures similar to those of the present study (Figs. 19 to 26). In metaphase asters (Fig. 22) the center is a mulberry-like, spheroidal structure of moderate size, not traversed by rays and having a definite contour; it "contains a group of irregular granules so as to give almost the appearance of a small nucleus in which the centrioles cannot be distinguished" (Wilson, '28, p. 677). In anaphase and telophase (Figs. 23 and 24) this relatively small and condensed body gives place to a very large vacular one which is spoken of as a centrosphere.

Boveri ('00), on the other hand, presents a very different picture of central bodies in cleavage-asters. His various plates show considerable variations of central body phenomena in different series. In all of them, however, there is a body at the center of each metaphase aster; it divides into two during the

astral history, keeping its identity as an individualized structure. One of his series is an almost diagrammatic presentation of such a sequence of events where in every stage there is a typical period-like centriole surrounded by a larger centrosome, about which are the rays; both centriole and centrosome divide, maintaining their identity throughout the process (Figs. 7 to 10). Boveri's figures have been reproduced by Buchner ('15, Fig. 17); by Hertwig ('23, Fig. 156); by Wilson ('28, Fig. 327); etc.

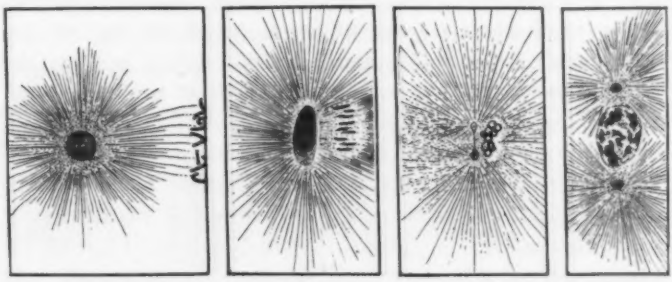


FIG. 7. FIG. 8. FIG. 9. FIG. 10.

Central bodies in cleavage-amphiasters of *Echinus* (Boveri, '00). Figs. 7 to 10 are reproduced from Boveri, '00, Plate V., Figs. 56, 58, 64 and 69. For discussion see pp. 107 and 120.

The following types of central bodies in echinoderm cleavage-amphiasters have been illustrated in other studies. A centriole (or centrioles) without a surrounding "centrosome" is shown by Kostanecki ('96) in *Echinus*. A centriole (or centrioles) surrounded by a "centrosome" (a configuration similar to that illustrated by Boveri ('00)) is shown by Hill ('95) and by Erlanger ('98) in *Sphærechinus*. A mulberry-like, large, spheroidal structure containing a number of granules (a configuration similar to that of Wilson ('95, '96, '00, '28)) is shown by Butschli ('92) in *Sphærechnius;* and by Wilson and Mathews ('95) in *Arbacia*. A clear, empty area at the center of the aster which may contain some scattered granules is shown by Ziegler ('04) in *Echinus;* and by Meves ('12) in *Parechinus*.

Whatever the structural details may be and whatever terminology is used by an investigator, it is widely taken for granted that central body behavior in echinoderm fertilization is typical of

central body behavior in fertilization generally. Boveri's paper ('00) developing his celebrated theory of the central apparatus was based as much upon investigations of *Echinus* eggs, as upon those of *Ascaris*. Yatsu includes Boveri's work on *Echinus* as one of the five cases "in which the centriole has been satisfactorily traced" in fertilization ('09, p. 371). Wilson cites central body behavior in the sea-urchin as a "typical case," mentioning it in this respect as in the same category with tunicates and nematodes ('28, p. 443, also footnote 4, p. 444). When various authors present generalized diagrams of fertilization, and of mitosis in cleaving eggs, some of them are based on the sea-urchin egg (Wilson, '28, Figs. 45, 46, 47 and 186; Hertwig, '23, Fig. 248; etc.). These diagrams are of course entirely schematic, but this is their very significance, since any phenomena delineated in such charts, whether they concern central bodies or asters or nuclei, present in diagrammatic form the most generally accepted ideas. The diagrams show a single central body (centriole) in the early sperm-aster; it divides into two, and they become the central bodies of the cleavage-amphiaster; they in turn later divide and a pair are passed to each daughter cell. Up to the present time, therefore, the central body phenomena of fertilized echinoderm eggs have been regarded as typical. Most investigators illustrate a minute period-like centriole which is frequently surrounded by a larger "centrosome." Not only are the central bodies of echinoderms assumed to give rise to the astral mechanism, but they are also supposed to maintain genetic continuity from cell to cell and from generation to generation.

The Relationship between Central Bodies and Astral Structure as Modified by Various Mitotic Phases in Fertilized *Echinarachnius* Eggs (Bouin's Fixation).

Method of Study.

A previous study of central bodies in cytasters of artificially activated *Echinarachnius* eggs (Fry, '28) proves that central bodies are present only if the rays satisfy two conditions: first, that they reach the center of the aster; second, that they are clear. Central bodies are always absent in cytasters if rays are very

vague, even though they do reach the astral center. They are also absent if the rays are clear peripherally but fail to reach the center. This invariable relationship between the occurrence of central bodies and the presence of well-formed rays reaching the center, holds good in cytasters no matter what are the modifications of astral structure caused by: (1) various intervals in its history; (2) various coagulation products produced by different fixatives; and (3) various effects of modifications of environmental factors. The conclusion is reached that the so-called central bodies seen in sections of fixed cytasters are nothing but the coagulated focal point of clear rays reaching the center and have no existence in the living cytasters as individualized entities.

The question arises, therefore, is it possible that central bodies of normally fertilized echinoderm eggs are likewise nothing but a coagulation product of the focal point of clear rays, having no existence in the living egg as individualized bodies? To investigate this problem there has been planned a series of five experiments with fertilized eggs of *Echinarachnius parma*. In each of them there are produced various modifications of astral structure, in order to observe whether or not central bodies occur only when clear rays reach the astral center and are always absent otherwise. In this group of experiments astral structure is modified by: (1) the various mitotic phases of the astral cycle; (2) different fixatives; (3) modifications of environmental factors; (4) differences in astral size during successive cleavages; and (5) hybridization. The first experiment on the effects of various mitotic phases is the subject of the present paper; the others are in process of completion.

The present investigation of fertilization asters was carried out by the same method used in the study of cytasters (Fry, '28, pp. 387–392). Its assumptions are as follows: (1) The form of a cell component when coagulated may be like that of the living condition, but on the other hand, it may differ to a greater or lesser degree. In how far the coagulation product is an "artifact" of the living component must be very carefully considered. (2) Within any one fixative, at each significant interval, it is necessary to study a large number of cells, so as to secure an adequate random sample. (3) They must be chosen at random, in such a way

as to avoid all unconscious selection of certain types. (4) Every part of the component, in each cell studied, should be accurately measured, and observations should be made concerning its physical structure. The surrounding related structures should also be analyzed in the same detail. This should be done in tabular form so that every individual is carefully checked with reference to each varying factor. The tabular form is necessary, otherwise omissions are made when analyzing many cells each with respect to a large number of points. If the component occurs in more than one section, it is necessary to study all the serial sections involved. In making certain measurements it is necessary to know in what plane the component has been cut. (5) The percentage of each class, at each interval, is accurately ascertained. Only those individuals are included within a class that are very similar with reference to all the variables. Unusual combinations of the variables are listed separately so as not to pass by small but possibly significant groups by paying attention only to the larger ones. (6) In compiling the data for any one fixative, all classes at all intervals are taken into account and none are omitted. (7) Whatever the abnormalities introduced by a fixative, at least that is a constant throughout the various intervals of that series. If significant relationships are apparent, they probably represent similar relationships in the living condition, despite any abnormalities introduced by fixation. (8) This technique should be repeated in a similar manner in a group of diverse fixatives. If a conclusion is reached that takes into consideration the relationships apparent in them all, it is probable that the results are valid for the living component, although they must be used with great caution. (9) It is assumed that the investigator takes into consideration any data concerning the chemistry of the coagulation products. (10) It is also taken for granted that he uses every means at his disposal to study the component in the living condition.

In the present investigation each sperm-aster was measured and analyzed with reference to about twenty items, listed in Table I. The cleavage-amphiasters were studied in like manner to analyze the interrelationships of their parts. About a hundred asters were so studied at each of nine intervals after fertilization (5

min., 7.5, 10, 15, 30, 45, 1 hr., 1 hr.–15 min., 1–30). The temperature of the living eggs was 20° C.

The results of the investigation, therefore, are based upon an exceedingly detailed analysis of about nine hundred asters. The method involves but the simplest quantitative principles as applied to cytological research. At each significant interval there is studied a sufficiently large number of individuals chosen at random. Each one is accurately measured and observations are made concerning a number of varying factors. The relations are noted. All classes are considered in arriving at the conclusion.

RAYS

Length:

In that region of the aster between edge of egg and male nucleus (designated as "male side").

In that region of the aster on the opposite side, toward female nucleus (designated as "female side").

Physical Structure:

Vague or clear? at "male side"; at "female side."

Delicate or coarse? at "male side"; at "female side."

Separate or close? at "male side"; at "female side."

Straight or crooked? at "male side"; at "female side."

Where are rays of early aster focused with respect to head and middle-piece of sperm?

CENTRIOLE

Is the bi-lobed granule present? Where located?

Are cytoplasmic granules present? How many? Size? Where located?

Is there any evidence of a true centriole?

CENTROSOME

(Any structure at the center of the aster differentiated from the ray area, other than granules and nuclear material.)

Width: at "male side"; at "female side."

Physical Structure:

Granular, corpuscular, etc.? at "male side"; at "female side."

Traversed by rays? at "male side"; at "female side."

Delimited from ray area? at "male side"; at "female side."

How stained in contrast to ray area? at "male side"; at "female side."

TABLE I.

LIST OF STRUCTURAL DETAILS WITH REFERENCE TO WHICH SPERM-ASTERS WERE ANALYZED (cf. Figs. 11 to 18).

Cleavage-amphiasters were measured and analyzed in a similar manner. For discussion see p. 112.

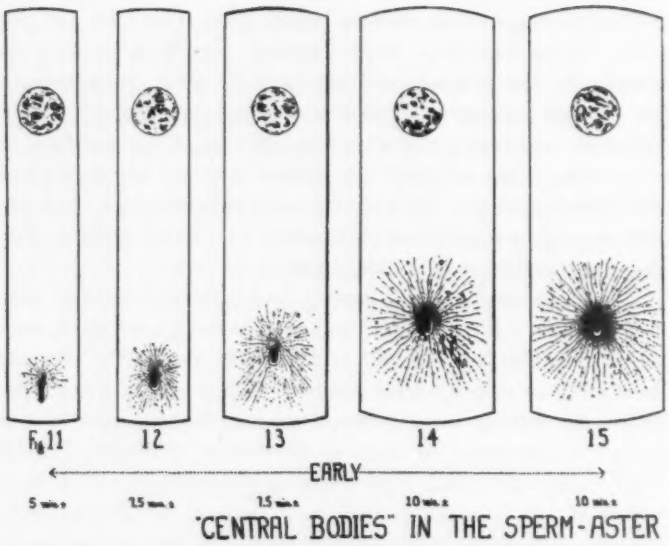
The drawings of the various classes (Fig. 11 to 26) are not each a delineation of a single "typical" or "best" cell, as is usually the case in cytological illustrations. Each dimension and the physical appearance of each part is an average of all the observations concerning that structure, made in all the members of that class. Thus, although the figures have the value of usual illustrations, and are "typical," they also have the value of charts that show the interrelations of a number of varying factors. The eggs were studied at 750 × magnification.

The eggs were fixed with Bouin's fluid (saturated aqueous solution of picric acid, 75 parts; formol, 25 parts; glacial acetic acid, 5 parts). This was selected because it is one of the reagents most effective in coagulating asters in such a manner as to show clear and distinct rays. It is certain that living asters have a radiate configuration at least in their outer portions. This is clearly seen when using water-immersion objectives of high magnification. It is probable that such reagents as Bouin's fluid coagulate asters with a minimum distortion of their radiate structure, although it must be kept in mind that the detailed structure of the coagulated rays may be quite different from that of the living ones. The eggs were sectioned 5 μ thick, and stained with Heidenhain's hæmatoxylin.

History of the Sperm-aster.

During the early history of the sperm-aster when it is small, the rays are delicate and are similar in structure on all sides (Figs. 11 to 15). During its middle history, when the aster reaches its maximum size and fills the egg, the rays become quite coarse on the side between the male chromatin and the edge of the egg ("male side") but remain delicate in the opposite portion ("female side") (Figs. 16 and 17). During the late history, when the nuclei fuse, the rays suddenly become very faint in all parts of the aster, although they still fill the egg, and continue to maintain a difference as to clarity between "male side" and "female side" (Fig. 18).

A darkly-staining, roughly-granular area is present at the center of the aster only at those times when the rays are coarse. It does not exist either at the beginning or at the end of the astral cycle



The illustrations are 650× enlarged. In each figure every dimension and the physical structure of each part is an average of all the observations made in a large number of eggs belonging to that class (cf. p. 110).

The diffuse granular "central body" is present only during that period of the astral cycle when rays are coarse, and only in that portion

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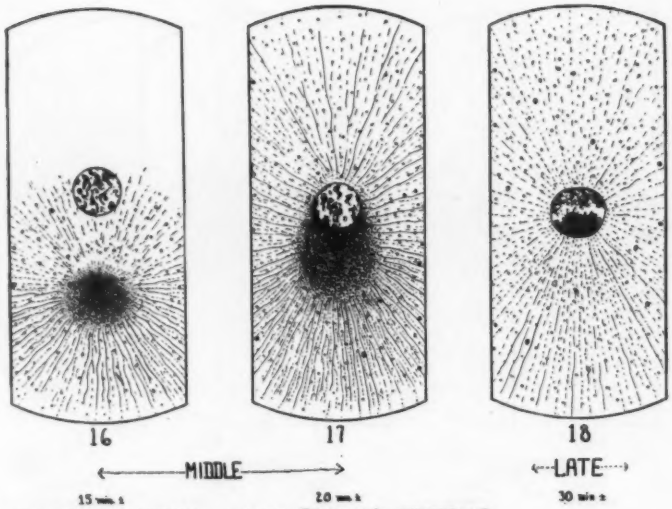
when they are delicate. Furthermore, during the period when it is present, it exists only on the "male side" where there are coarse rays, and it is absent (or is occasionally present only to a very slight extent) on the "female side" where rays remain delicate. It is further to be noted that the size of the central granular area is proportional to that of the aster. In about four hundred sperm-asters studied by the exceedingly detailed method outlined above, there are no exceptions to the correlation between the presence of coarse rays and the presence of a central granular configuration; and between the absence of coarse rays and the absence of such a configuration. It appears and disappears when and where coarse rays appear and disappear. This suggests the conclusion that the granular center (centrosome) is nothing but a coagulation product of heavy rays at the inner ends where they converge.



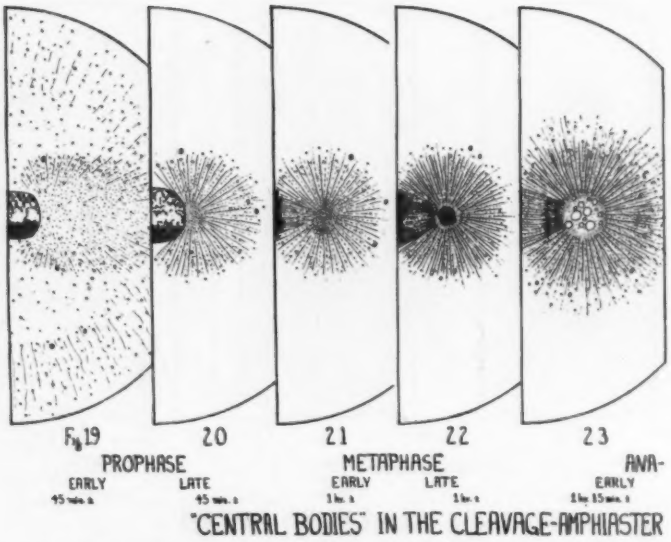
of the aster (the "male side") where they are coarse. The "central body" appears and disappears as heavy well-formed rays appear and disappear. It is a coagulation artifact of rays substance at the astral center produced only when rays are coarse. For discussion see pp. 113 and 118.

The entire aster, including the central area, always contains a number of the deeply-staining granules of various sizes that are abundant throughout the cytoplasm. If, as occasionally happens, there are but one or two at the center, such a configuration simulates a centriole, single or double, surrounded by a vague type of centrosome, but it is without significance.

As noted above, the bi-lobed granule of chondriosome material, introduced by the sperm's middle-piece, is present only during the first ten minutes of the astral history (Figs. 11 to 15). Thereafter it wanders out into the cytoplasm.

History of the Cleavage-amphiaster.

During the earliest and latest stages of the cleavage-amphiaster, *i.e.*, in early prophase and late telophase (Figs. 19 and 26), the rays are exceedingly vague, and the physical structure of the

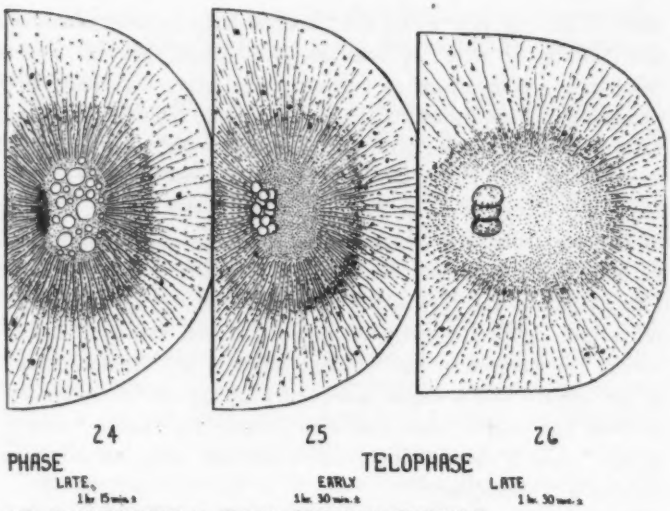


The illustrations are 650 × enlarged. In each figure every dimension and the physical structure of each part is an average of all the observations made in a large number of eggs belonging to that class (cf. p. 110).

The "pleuricorpuscular central body" which enlarges into the "centrosphere" is present only during that period of the astral cycle

central area at both times is like slightly vacuolated cytoplasm. There are two periods when the rays are clear though very delicate; the first of them is during late prophase and early metaphase (Figs. 20 and 21); the second is during early telophase(Fig. 25). During both these periods the central area is granular and not delimited. Only during the aster's middle history, from late metaphase to late anaphase, are the rays coarse (Figs. 22 to 24), and only at this time does there exist the so-called central body. At first, during late metaphase (Fig. 22), it is a small darkly-staining, mulberry-like structure, like a cluster of closely-aggregated vacuoles. During the anaphase stages (Figs. 23 and 24) it enlarges as the aster enlarges, and becomes a lightly-staining vascular area, the echinoderm "centrosphere."

The central body structures of cleavage-amphiasters, like those of sperm-asters, exist only when rays are coarse; they are absent



when rays are coarse. It appears and disappears as heavy well-formed rays appear and disappear. It enlarges and changes shape as the aster enlarges and changes shape. It is a coagulation artifact of ray substance at the astral center produced only when rays are coarse. For discussion see pp. 115 and 118.

when rays are delicate. They appear and disappear as coarse rays appear and disappear. This suggests the conclusion that the central bodies of cleavage-asters, as in the case of sperm-asters, are not individualized entities, but are nothing but a coagulation product of heavy rays at the inner ends where they converge.

In about five hundred cleavage-asters studied in the detailed manner previously described, there were found no exceptions to the correlation between the presence of coarse rays and the occurrence of a central body. This is strikingly shown in comparing early metaphase asters with late ones (Figs. 21 and 22). They are similar as to size; in both, the chromosomes are at the midpoint of the spindle; they differ only in the fact that the earlier stage has delicate rays and the later one has coarse rays. The former has no central body; the latter has a fully-formed central body. In over a hundred and fifty such metaphase asters studied,

there is not one case where the "central body" exists when rays are delicate, or where it does not exist when rays are coarse. Similarly, a comparison of late anaphase asters with early prophase ones (Figs. 24 and 25) shows the same relationship. Both are of maximum size; both are elongate at right angles to the spindle axis; both have asters with inner and outer zones; in both, the chromosomes occur at the inner edge of the spindle, existing as vesicles (small and numerous in the early stage; larger and partially fused in the later one). The only difference between the asters concerns the rays which are heavy and coarse in late anaphase, but delicate in early telophase. A well formed "centrosphere" is present in the former; there is no "centrosphere" in the latter. Not a single case occurs among the asters studied in these two stages where one with delicate rays has a "centrosphere"; nor is there a single case where one with coarse rays is without a "centrosphere."

In the cleavage-amphiaster the rays are coarser and straighter and lie more closely together than is the case in the sperm-aster. All cytoplasmic granules, with but rare exceptions, are completely excluded from the central area and from among the inner portions of the rays. They occur occasionally among the tips of the rays and are very abundant in the surrounding cytoplasm. This situation is in strong contrast to the condition in the sperm-aster where granules are common between the rays throughout their entire length, as well as in the central area. In those very rare cases among cleavage-asters where one or two granules do occur at the center, they simulate centrioles, but are without significance.

Discussion.

The So-called Central Bodies in Fertilized Echinarachnius Eggs.

In *Echinarachnius* the conditions under which "central body" structures are present in cleavage-amphiasters are very similar to those necessary for their presence in sperm-asters. In both, they are present only when rays are coarse, and are absent when rays are delicate; they appear and disappear with the appearance and disappearance of heavy rays. In both, the size of the configuration is proportional to that of the aster; in the case of the cleavage-amphiaster not only does the "central body" enlarge as the aster enlarges, but it changes shape as the aster changes shape

(Figs. 23 and 24). In both, the evidence is equally strong that the "central bodies" are nothing but the coagulated inner ends of heavy rays.

If "central bodies" of sperm-asters and cleavage-asters are produced by a similar mechanism, how are the differences in their structure explained? Comparing the situation with reference to rays, the contrasting factors are as follows: in sperm-asters rays are heavy, but are not as coarse as in cleavage-asters; in sperm-asters they are farther apart than in cleavage-asters, as shown by the presence of cytoplasmic granules between the rays of the former and their absence among those of the later. The most important difference, however, between the two types of asters, concerns whether or not the focal center is occupied by nuclear material. In the sperm-aster there is a nucleus (whether male alone, or male and female; whether separate or fused), whereas in the cleavage-asters there is no nuclear material at the center during the period when "central bodies" are present. These differences probably account for the morphological differences between "central bodies" of sperm-asters and those of cleavage-asters.

The behavior of "central bodies" in nuclear asters of fertilized echinoderm eggs is in complete harmony with central body phenomena in cytasters of artificially activated ones (Fry, '28). In both of them, the so-called central bodies appear and disappear with the appearance and disappearance of coarse rays; in both, they are the coagulated center where the rays converge.

It is proved in the case of fertilized eggs of *Echinarchnius* that the asters form "central bodies" only when rays are coarse. Furthermore, it is probable that this occurs only as a result of coagulation, and that in the living condition the "central body" is actually nothing but ray substance at the astral center. Live asters have been carefully studied at high magnifications with water-immersion objectives. A radial configuration can be seen distinctly in the outer parts. The central areas are perfectly structureless and hyaline, except for the possible presence of nuclear material. Were the "central bodies" seen on slides of coagulated asters, actual structures present in the living condition, it is at least probable that they would be visible since they are larger than nuclear structures than can be seen.

The usual hypotheses concerning the mitotic rôle of central bodies in animal cells (pp. 101–104) are shown to be meaningless in the case of fertilized eggs of *Echinarachnius*. They are not the "dynamical centers," the "formative foci," the "most persistent element of the astral mechanism," nor have they genetic continuity. *It is probable that the so-called central bodies of fertilized Echinarachnius eggs are nothing but coagulation artifacts.*

The So-called Central Bodies in Fertilized Echinoderm Eggs.

"Central body" phenomena in *Echinarachnius* are probably typical of echinoderms generally. The general features of the illustrations of the present study (Figs. 11 to 26) are very similar to those of previous investigations. The differences that occur with respect to the detailed structure of central bodies are explained by the use of different fixatives which produce various differences in the coagulation products of the rays and hence form various types of central bodies. The effects of different coagulation agents is now under investigation and the results will be reported in the next paper of the present series. In those cases where the central bodies of sperm-asters are illustrated as large homogeneously-granular structures without a period-like centriole (Wilson '95, '96; Wilson and Leaming '95; Wilson and Mathews '95; Ziegler '04) the coagulation products are similar to those of the present study. In cases where there is a period-like centriole, or centrioles (Hill '95; Kostanecki '96; Erlanger '98; Wilson '00, '01, '23, '28), the situation is probably explained by the fact that those asters which happen to contain one or two cytoplasmic granules at their mid-point were selected as "normal" whereas the others were dismissed as "poorly fixed"; or such "centrioles" may be small clearly-delimited coagulation products of the focal point of astral rays. (The study that is now in progress dealing with the effects of various fixing agents upon "central bodies" shows that they may vary from large vaguely-delimited diffuse types to small condensed delimited structures, depending upon the fixative that is used.) The misinterpretation by Boveri ('00) of the bi-lobed granule has already been noted. The wide variety of central body struc-

ture in cleavage-amphiasters is likewise probably explained by different coagulation products, coupled with a misinterpretation of cytoplasmic granules that sporadically occur at the astral center. It is possible, of course, that the central body phenomena of *Echinarachnius* are not typical of echinoderms. Furthermore, modifications due to species differences must be kept in mind. Nevertheless, it is probable that the present study is valid for echinoderms generally.

Boveri ('97) reports a case in *Echinus* eggs where at the first cleavage an isolated central body passes into one blastomere, whereas all the nuclear material and the other central body remains in the other blastomere. The isolated center is described as dividing synchronously with the other. This observation is given considerable theoretical importance by Wilson ('28, p. 441). If the results of the present paper are valid and generally applicable to echinoderms, there is some source of error in Boveri's observations. The discrepancy awaits further study, but it is to be noted that he worked with eggs that had been shaken. The supposedly enucleated blastomere may have contained some scattered nuclear material that was invisible.

Hypotheses Concerning the Mitotic Rôle of Central Bodies in the Light of the Present Investigation.

The assumption that central bodies are the formative foci of the astral mechanism, and the hypothesis that they maintain genetic continuity from cell to cell, are well illustrated in many species during the final divisions of the germ cells. The question arises, however, to what extent can their behavior at this period be regarded as typical? It is an accepted biological principle that the foundamental phenomena of any one type of cell will probably be found to be true of many other cells. This is a very dangerous assumption, however, to apply to the rôle of cell components during spermatogenesis. The behavior of the chromosomes with reference to synapsis and reduction, the behavior of the Golgi-bodies in giving rise to the acrosome, the behavior of the chondriosomes in forming the nebenkern, are not thought of as typical of those components generally. The same is true of the behavior of central bodies in producing the axial filament. In how far,

therefore, can other phases of central body behavior occuring during gametogenesis be regarded as typical? Is it safe to assume that because they give rise to asters and maintain genetic continuity during this very specialized phase of cellular history, that therefore this is probably typical of their mitotic rôle in most cells? The fact that central bodies occur in higher plants only in the final divisions of the spermatozooid-forming cells, indicates that their behavior during the formation of motile gametes generally may be peculiar to that period. Their chief function at that time is probably that of a blepharoplast. In any event it is very unsafe to reason from any phase of central body behavior during the history of germ cells to their behavior in fertilization and mitosis generally.

The conclusion of the present study is that the so-called central bodies in fertilized eggs of *Echinarachnius* are nothing but coagulation artifacts. To what extent this conclusion may be true of central bodies in other species awaits further study by the same method used in this investigation. There are various facts indicating that the present result may have wide-spread application to central bodies in fertilization and in mitosis (with the exception of the final divisions of motile germ cells). It is well known that central bodies occur only in connection with asters; they are absent in higher plants (except in spermatozooid formation) where asters are absent. There are many cases in animal cells where the central bodies disapear at certain stages in the cell history and later reappear; they disappear as the asters fade, and reappear as the asters reform. In those animal eggs where polar bodies are formed by a spindle without asters, there are no central bodies at the ends of the spindle although they are present in the same eggs at the center of the sperm aster. It is frequently stated that it is "difficult to fix" central bodies during that stage of fertilization, the so-called "pause," after the sperm-aster has faded and before the cleavage-asters have become well-formed. These facts indicate that it is not an unreasonable suggestion that the result of the present study may have wide application. In many cases central bodies of fertilization and mitosis (except in gametogenesis) may have no existence as individualized structures, and may be nothing but coagulation artifacts produced by

fixation of ray material at the center of asters having clear rays. This idea is put forward tentatively as a working hypothesis only. It is possible, of course, that central body phenomena of fertilization and mitosis may not fall under one category of behavior. At least the present study raises serious doubts concerning the usually accepted theories.

RÉSUMÉ.

1. The present status of the hypotheses concerning the mitotic rôle of central bodies in the animal cell may be summed up as follows. There is general agreement that they are "division centers," the formative foci about which the astral mechanism arises and its most persistent element. There is difference of opinion as to whether or not they maintain genetic continuity from cell to cell and from generation to generation, but there is a tendency in some quarters to tentatively accept such a hypothesis as a fruitful working assumption.

2. Much confusion exists in the literature concerning the terminology and relationships of the central body. Its most constant and essential component is a minute period-like *centriole*. This is usually surrounded by a larger structure, the *centrosome*, about which is the ray area. The term "centrosome," however, is used in widely different ways by various authors. Furthermore, in some cases it is often difficult to determine whether the central apparatus is a "centriole" or a "centrosome." These ambiguities led to the adoption of the more inclusive and non-commital term *central body*. This is historically the older and leaves open the question as to precise homologies in any particular case.

3. There is a wide variety in the structure of echinoderm central bodies as reported by various investigators. The majority of studies illustrate a minute period-like centriole which is usually surrounded by a larger structure, the centrosome. Whatever the structural details may be in any given study, and whatever terminology is used, it is taken for granted that central body behavior in echinoderms is "typical." Echinoderm central bodies are assumed to give rise to the astral mechanism, and it is regarded as highly probable that they maintain genetic continuity from cell to cell and from generation to generation.

4. In the present investigation about nine hundred asters in the

eggs of *Echinarachnius parma* were studied at various stages during the mitotic cycle from fertilization to first cleavage. They were fixed in Bouin's fluid, a reagent that clearly coagulates rays. Each aster was measured and analyzed with reference to about twenty points concerning its rays and central bodies. The study was conducted upon the following assumptions: in the cytological investigation of a cell component it is necessary to study large numbers of cells at each interval of significant change; to choose them by a method that prevents all unconscious selection of certain classes; to analyze and measure each individual with reference to the maximum number of factors; to determine relations; and to take all classes into consideration in arriving at the result. The method involves only the simplest quantitative principles as applied to cytological research.

5. Such a quantitative analysis of about four hundred sperm-asters shows that a granular configuration ("centrosome") is present at the astral center only during its middle history when rays are coarse, and that it is absent at the beginning and at the end of the cycle when rays are delicate. Its size is proportional to that of the aster. It appears and disappears as coarse rays appear and disappear.

6. A similar analysis of about five hundred cleavage-asters shows that the "pleuricorpuscular" echinoderm central body which enlarges into the "centrosphere," is present only during the middle-history of the aster when rays are coarse, and is absent at the beginning and at the end of the cycle when rays are delicate. Its size and shape are proportional to that of the aster. The central body appears and disappears as coarse rays appear and disappear.

7. The conclusion is suggested, therefore, that the so-called central bodies of both sperm- and cleavage-asters are not individualized entities giving rise to the asters, but that they are formed by the aster only when rays are coarse. Furthermore, it is probable that the aster forms the "central body" only when it is coagulated. Live asters were carefully studied at high magnifications with water-immersion objectives. A radial configuration is distinctly visible in the outer parts; the central areas are perfectly hyaline, except for the possible presence of nuclear material.

Were the so-called central bodies of coagulated asters, actual structures present in the living condition, it is at least probable that they would be visible, since they are larger than nuclear structures that can be seen.

8. The behavior of "central bodies" in nuclear asters of fertilized *Echinarachnius* eggs is in complete harmony with central body phenomena in cytasters of artificially activated ones (Fry, '28). In both, the so-called central bodies appear and disappear as coarse rays appear and disappear; in both, they are the coagulated center where well-formed rays converge.

9. The usual hypotheses concerning the mitotic rôle of central bodies in animal cells are meaningless in the case of *Echinarachnius* fertilization. The so-called central bodies of fertilized *Echinarachnius* eggs are nothing but coagulation artifacts. The situation in this species is probably typical of echinoderms in general, as the phenomena of the present study are very similar to those illustrated by previous students of echinoderm fertilization. It is probable that the central bodies previously described in echinoderm fertilization are either cytoplasmic granules that happen to be at the center of the aster or that they are nothing but the coagulated focal point of rays. Differences in structural details, as reported by various investigators, are probably due to the effects of various fixatives.

10. The assumption that central bodies are the formative foci of the astral mechanism, and the hypothesis that they maintain genetic continuity from cell to cell, are well illustrated in many species during the final divisions of the germ cells. The behavior of all cell components, however, during gametogenesis, including that of central bodies, is in many ways different from their usual rôle. It is very dangerous, therefore, to expect that the phenomena found during this specialized phase of cell history may be typical of cells generally. It is very unsafe to reason from central body behavior during gametogenesis to their behavior in fertilization and mitosis.

11. The conclusion of the present study that "central bodies" in fertilized *Echinarachnius* eggs are nothing but coagulation artifacts may possibly have wide-spread application to the rôle of central bodies in fertilization and mitosis generally (with the

exception of the final divisions of motile germ cells). It is well known that central bodies occur only in connection with asters; they are absent in higher plants (except in spermatozooid formation) where asters are absent. There are many cases in animal cells where the central bodies disappear at certain stages in the cell history and later reappear; they disappear as the asters fade, and reappear as the asters reform. In those cases where polar bodies are formed by spindles without asters, central bodies are absent from the ends of the spindle although the same cell contains a central body in the sperm-aster. They are "difficult to fix" during the "pause" in fertilization, after the sperm-aster has faded and before the cleavage-asters have become well-formed. Hence it is not an unreasonable tentative suggestion that the results of the present study may be found to have wide application, and may fundamentally modify current hypotheses concerning central bodies in fertilization and mitosis.

LITERATURE CITED.

Agar, W. E.
'20 Cytology. Macmillan and Co., Limited, London.
Boveri, T.
'97 Zur Physiologie der Kern- und Zellteilung. Sitz.-Berichte der Phys.-med. Ges. Würzburg.
'00 Zellen-Studien. Heft 4. Ueber die Natur der Centrosomen. Jen. Zeitsch., 25.
Brachet, A.
'17 L'œuf et les facteurs de l'ontogénèse. Octave Doin et Fils, Paris.
Buchner, P.
'15 Praktikum der Zellenlehre. Gebrüder Borntraeger, Berlin.
Bütschli, O.
'92 Untersuchungen über microscopische Schäume und das Protoplasma. English translation by E. A. Minchin. Adam and Charles Black, London.
Doncaster, L.
'20 An Introduction to the Study of Cytology. The Cambridge University Press.
Erlanger, von R.
'98 Zur Kenntnis der Zell- und Kernteilung. II. Ueber die Befruchtung und erste Teilung des Seeigel-Eies. Biol. Cent., 18.
Field, G. W.
'95 On the Morphology of the Echinoderm Spermatozoön. Jour. Morph., 11.
Flemming, W.
'91 Neue Beiträge zur Kenntniss der Zelle, II. Arch. mikr. Anat., 37.

Fry, H. J.

'28 Conditions Determining the Origin and Behavior of Central Bodies in Cytasters of *Echinarachnius* Eggs. BIOL. BULL., 54.

Hertwig, O.

'23 Allgemeine Biologie, seventh edition. G. Fisher, Jena.

Hill, M. D.

'95 Notes on the fecundation of the egg of *Sphærechinus granularis* and on the maturation of the egg of *Phallusia mammilata.* Quart. Journ., 38.

Just, E. E.

'27 History of the Middle-piece of the Spermatozoön in the Fertilized Egg of *Echinarachnius parma.* Anat. Rec., 37. (Abstract, p. 162.)

Kostanecki, K.

'96 Ueber die Gestalt der Centrosomen im befruchteten Seeigelei. Anat. Hefte, 22.

Lillie, Frank R.

'19 Problems of Fertilization. The University of Chicago Press.

Lillie, Frank R., and Just, E. E.

'24 Fertilization. This discussion is Chapter VIII of General Cytology, edited by Edmund V. Cowdry. The University of Chicago Press.

Meves, R.

'12 Verfolgung des sogenannten Mittelstückes des Echinidenspermiums im befruchteten Ei bis zum Ende der ersten Furchungsteilung. Arch. mikr. Anat., 80.

Sharp, L. W.

'26 An Introduction to Cytology. The McGraw-Hill Book Co., New York.

Wilson, E. B.

'95 Archoplasm, Centrosome and Chromatin in the Sea-urchin Egg. Jour. Morph., 11.

'96 The Cell in Development and Inheritance, first edition. The Macmillan Co., New York.

'97 Centrosome and Middle-piece in the Fertilization of the Egg. Science, 5.

'99 On Protoplasmic Structure in the Egg of Echinoderms and Some Other Animals. Jour. Morph., 15, suppl.

'00 The Cell in Development and Heredity, second edition. The Macmillan Co., New York.

'01 Experimental Studies in Cytology. II. Some Phenomena of Fertilization and Cell-division in Etherized Eggs. Arch. f. Entw.-mech., 13.

'23 The Physical Basis of Life. Yale University Press.

'28 The Cell in Development and Heredity, third edition, 1925, with corrections. The Macmillan Co., New York.

Wilson, E. B., and Leaming, E.

'95 An Atlas of the Fertilization and Karyokinesis of the Ovum. The Macmillan Co., New York.

Wilson, E. B., and Mathews, A. P.
'95 Maturation, Fertilization and Polarity in the Echinoderm Egg. Jour. Morph., 10.
Yatsu, N.
'09 Observations on Oökinesis in *Cerebratulus lacteus*. Jour. Morph., 20.
Ziegler, H. E.
'04 Die ersten Entwickelungsvorgänge des Echinodermeneies insbesondere die Vorgänge am Zellkörper. Denkschrift med.-naturw. Gesell. Jena (Festschrift z. E. Haeckel).

PARTHENOGENESIS AND THE INHERITANCE OF COLOR PATTERNS IN THE GROUSE LOCUST *PARATETTIX TEXANUS* HANCOCK.

ROBERT K. NABOURS AND MARTHA E. FOSTER.[1]

Introduction.

Newell (1914) reported segregation of genetic factors in parthenogenesis, wherein hybrid Carniolan-Italian queen bees gave equal numbers, respectively, of pure Carniolan and Italian drones. Previously, Perez (1879) and Cuénot (1909) had noted variations among the drones from hybrid queens, but more than one pair of factors had apparently been involved. Observations were made in 1923–24, in the apiary of the Kansas Agricultural Experiment Station of the offspring of separate queen bees considered hybrid. The drones varied greatly in their characteristics, especially with respect to color, ranging from black, through various degrees to the yellow of the Italian. These queens thus exhibited a considerable degree of genetic complexity, indicating the presence of two or more independent pairs of factors for color and other characteristics.

Parthenogenesis in the grouse locusts was first recognized in 1915, in attempts to crossbreed males of *Paratettix texanus* with females of *Apotettix eurycephalus*. It was noted that the offspring from such matings were exclusively females, and carried the color pattern of the female of the pair if she were homozygous, or segregated into her component, or cross-over patterns if she were heterozygous. Then it was soon ascertained that *A. eurycephalus* females which had never been exposed to males of any kind behaved in these respects precisely as did those exposed to *P. texanus* males. As many as 4,470 females and seven males produced in parthenogenetic breeding, mostly from females which had not been exposed to males at all, many showing segregation and crossing-over of factors for color patterns, had been re-

[1] Contribution No. 110, Department of Zoölogy, Kansas Agricultural Experiment Station.

corded by August 1, 1918 (Nabours, 1919). Later, some females of *A. eurycephalus* were bred seven consecutive generations parthenogenetically, and there was further rather extensive experimental breeding of this species both bisexually and parthenogenetically (Nabours, 1925, 1929).

Segregation of factors for color patterns in parthenogenesis was found to occur also in *Telmatettix aztecus* (Nabours and Snyder, 1928). There have been a few females of *Tettigidea parvipennis pennata* produced in this laboratory from unmated parents. One unmated individual of this species of the genetic composition F/M, (for color patterns see Bellamy, 1917), gave two F/F and three M/M females (1922, unpublished).

Whiting (1921a) reported on the wasp *Hadrobracon brevicornis*, wherein the unmated females, hybrid for black and orange eyes, gave nearly equal numbers, respectively, of black and orange eyed males. He also reported a factor for lethal (Whiting, 1921b) which exhibited 19.5 per cent. of crossing over with the factors for orange and black eyes in parthenogenetic breeding.

Hybrid moths from the crossing of *Tephrosia bistortata* males with *T. crepuscularia* females reproduced parthenogenetically, and there was segregation with respect to wing color and pattern. The unmated females of neither species alone would give progeny. In connection with the report of these results, the authors (Peacock and Harrison, 1925) advanced the *hypothesis that parthenogenesis was consequent upon hybridity*. In a later paper (1926), these investigators adduced from my tables (Nabours, 1919, 1925) that all the parthenogenous females of the grouse locust *A. eurycephalus* had been hybrids from the crossing of one variety of the species from Tampico, Mexico, with another from the region of Houston, Texas. They welcomed this as evidence constituting strong confirmation of their hypothesis.

Progeny have resulted in this laboratory in three of eight attempts to cross males of *A. eurycephalus* with females of *P. texanus*, once in 1912 and twice in 1915. In the first case, the female, one generation from Many, Louisiana, gave five females, all appearing to be like herself. In the other cases, an I/P female gave one I/I, or +/I, and two P/P, or +/P, females, and a B/C female gave two C/C, or +/C, females. Both these parents

were one generation from Houston, Texas. Since these three females had been probably also exposed to brothers, and as none of the progeny was tested, the results had been regarded with doubt. However, a reëxamination of the records, in the light of further experience, strengthens the possibility that these were also cases of parthenogenesis.

Females which are exposed to males of any kind are considered as having reproduced parthenogenetically (1) if the contrasting dominant characteristics of the males do not develop in the offspring, (2) if practically all the offspring are females, or (3) if the offspring, when bred further, are found to be homozygous for those factors for which they should be heterozygous had the eggs been fertilized. At least two, or sometimes all of these criteria are applied in the determination of each case. It is obvious that, in those matings where the males give some or all recessive gametes, a proportionate number of the female progeny which are not tested by further breeding, or determined histologically, may possibly be, in fact, of parthenogenetic origin.

Parthenogenesis in *Paratettix texanus*.

Parthenogenesis in *Paratettix texanus* was first definitely recognized on November 14, 1922, when the offspring of a pair of heterozygous individuals of contrasting dominant color patterns exhibited segregate patterns of the female only, and all were females (Table II., item 1). During the five years following, until November, 1927, 32 other females that had been similarly exposed to males of contrasting dominant color patterns produced 187 recorded females and one male, all bearing exclusively the maternal characteristics, Table II., items 2–17, 30, 32–34, 36, 40, 44–50, 52, 79 and 81. Of these offspring, 26 were successfully tested in matings, or by reproducing again parthenogenetically, and all were found to have been homozygous for the dominant maternal patterns, Table II., and V.

Some of the males to which the 33 females had been exposed were thought to have been impotent, while the others died, or escaped from the cages before matings occurred. In both *Apotettix curycephalus* (Nabours, 1919, 1925, 1929) and this species, *P. texanus*, it has appeared that any female, if potent at all, re-

produced bisexually, rarely, if ever parthenogenetically, when mated with a potent male.

These 33 cases of parthenogenesis among females exposed to males have occurred during the last quarter of a period of the breeding of *P. texanus* covering nineteen years, although there had been many opportunities for parthenogenesis from the beginning. In numerous cases males had died, or escaped from the cages after the matings had been made, and the females remained for long periods without offspring ensuing. The matings and records of the entire period have been made in such a manner that, with comparatively few exceptions, parthenogenesis would have been indicated.

Following the observations of the first few cases of occasional parthenogenesis among females that had been exposed to males, and having in mind the experiences with *A. eurycephalus* (Nabours, 1919, 1925, 1929) a number of females of *P. texanus* were separated from the males. From 75 of these which were not exposed to males after becoming adult, 625 offspring were secured and transferred from the parent to the progeny cages. Of these, 393 females and one male became large enough to permit records being made of their sex and color patterns, and there were seven the sex of which was not noted (Table II.).

Materials and Methods of Breeding.

The specimens used in these experiments were taken from the stocks of *P. texanus* which had been bred in the greenhouse, beginning with individuals secured at Houston, Texas, in September, 1908 (Table IV.). New specimens had been added nearly every year, during the first 13 years, or until 1921, from Houston, San Antonio, Sugarland, Mackay and Beaumont, Texas, and Many and Baton Rouge, Louisiana, but principally from Houston and Sugarland. Specimens were secured and added to the stocks during the last five years from San Antonio, Houston, Sugarland, and, in addition, from Austin and College Station, Texas. Therefore, there has been abundant opportunity for hybridization of probably slightly differing varieties, a feature which will be discussed farther on.

Most of the elementary color patterns of *P. texanus*, and some

of their hybrid complexes, have been previously approximately described and illustrated (Nabours, 1914, 1917, 1923, and Plate III., 1929). The full list of those employed in experimentation up to date may be described, though inadequately, as follows: (1) +/+ (old AA), mottled gray, a pattern common to practically all the grouse locusts, and now considered the normal recessive, or "wild type"; (2) B/B, white over pronotum and parts of posterior femora; (3) C/C, white anterior pronotum, posterior dark or mottled, reddish brown legs; (4) Cext/Cext, the same as C/C, but with an extension of the white of the anterior pronotum posteriorly, and the line between the white anterior and dark posterior is not sharp; (5) Cof/Cof (old QQ) practically the same as C/C, but with red middle legs and conspicuously orange colored femora of the jumping legs; (6) D/D, the same as +/+, but with conspicuous white spots on hind femora; (7) E/E, broad yellow stripes along median pronotum and on distal ends of posterior femora; (8) F/F, broad mahogany stripes along median pronotum and posterior femora; (9) S/S, broad yellowish gray, nearly white stripes along median pronotum and on distal ends of hind femora; (10) Sm/Sm (old ISIS), broad brown, slightly red stripes along median pronotum and on distal ends of posterior femora (distinctly different from the other stripes and the only mutant observed to occur in the greenhouse); (11) S_1/S_1, broad nearly clear white stripes along median pronotum and on distal ends of hind femora, and with red middle legs; (12) P/P, broad brown stripes along median pronotum and on distal ends of posterior femora; (13) L/L, trilineate, three nearly white lines along the pronotum and one along femora of hind legs; (14) K/K, narrow white stripe along median pronotum, and red middle legs, almost indistinguishable from K/K, of *Apotettix eurycephalus,* (Nabours, 1925); (15) J/J, conspicuous large white spot over broad part of pronotum, identical with Y/Y in *A. eurycephalus* (loc. cit.); (16) Jof/Jof, the same as J/J, but with prominently orange colored posterior femora and red middle legs; (17) H/H, large yellow, or orange spot covering the same area as the white spot of J/J; (18) Hm/Hm, a gray, slightly orange spot, covering the same area as the spot H/H; (19) I/I, a dark mahogany spot over the same area as that of J/J; (20) M/M,

brown all over pronotum. Hybrid M/S looks precisely like Sm/Sm. It is now thought, contrary to the previous idea, that the origin of Sm was due to the mutation of a gene closely linked with the S gene (Nabours, 1917, pp. 48, 52, 53); (21) N/N, a brown gray all over; (22) N_1/N_1, dull orange, or henna all over; (23) N_2/N_2, brilliant orange all over. These first named twenty-two factors for color patterns are extremely closely linked. Hm is the only one to have crossed over at all. Some of them may be actually multiple allelomorphs. (24) θ/θ, dense black over anterior pronotum, fading somewhat towards the posterior, and extending over the hind femora; (25) sf/sf, white spots on posterior femora, resembling D/D, but recessive in heterozygotes, and not showing well, even in homozygous condition, with some of the dominant patterns, as C, Cof, Jof; (26) ϕ/ϕ, reddish, or pink all over, hardly discernable, almost recessive in heterozygotes. These two, sf/sf and ϕ/ϕ, are the only colors so far discovered in all the grouse locusts, except the normal recessive, +/+, that can in any sense be considered recessive, and they are only partially so. These last described three are extremely loosely linked with each other and all the others, or they may be on separate pairs of chromosomes (For θ, see Haldane, 1920).

The breeding methods have been about the same as those employed in all the experiments with the grouse locusts (loc. cit.). A longer time is required to obtain offspring from unmated females, and they are fewer in numbers, than from mated ones. A comparison of the productivity of unmated individuals with their mated sisters is shown in Table I, covering the period, January–October, 1925. This comparison shows that 46.6 per cent. of the unmated females were productive, while 62.5 per cent. of their mated sisters gave offspring. The average number of offspring for the unmated individuals was 9, while the average number hatched from the mated sisters was 60.13, or more than six times as many. Sixty-seven and five tenths per cent. of the offspring hatched from unfertilized eggs, against 53.75 per cent., of those hatched from the mated sisters, were recorded. The discrepancy in the proportions recorded, however, might not have been due so much to the greater viability of the parthenogenous progenies as to the care they were given, and early age at which

records were made. These were noted every day, while the progenies of the mated sisters were part of the larger breeding projects and were recorded only in their turn, which was sometimes long after they were large enough, and after considerable mortality.

Miss Isabel Potter has had a considerable share in conducting these experiments, and the principal task in the preparation of the tables.

TABLE I.

PRODUCTIVITY OF UNMATED FEMALES COMPARED WITH MATED SISTERS (JANUARY-OCTOBER, 1925).

Months Females Were Placed in Mating Jars.	Unmated Females.				Mated Females.			
	Number of Unmated Females.	Number Productive.	Number of Offspring Transferred.	Number of Offspring Recorded.	Number of Mated Sisters.	Number Productive.	Number of Offspring Transferred.	Number of Offspring Recorded.
January.....	1	1	24	17	2	1	128	66
February....	1	0	0	0	2	2	175	100
March.....	27	23	250	188	15	14	1,078	731
April.....	7	7	32	24	0	0	0	0
May.....	12	8	75	39	5	3	402	171
June.....	31	4	26	9	33	18	665	285
July.....	18	3	20	7	9	5	168	84
August.....	0	0	0	0	0	0	0	0
September...	8	3	14	14	5	2	90	17
October.....	0	0	0	0	1	0	0	0
Totals....	105	49	441	298	72	45	2,706	1,454
	46.6% productive; average 9. offspring; 67.5% recorded.				62.5% productive; average 60.13 offspring; 53.75% recorded.			

THE EXPERIMENTS.

The data are presented mainly in tables with explanations, and their use illustrated by a few succinctly elaborated examples. The tables have cross references so that the progenitors, or posterity, both males and females, of any individual, so far as they appear to be related to parthenogenesis, may be traced. Table II. shows the parthenogenetic breeding of 108 females, composing 83 items of individuals and groups. Thirty-three of the females, items 1–17, 30, 32–34, 36, 40, 44–50, 52, 79 and 81, had been exposed to males; the other 75 had not, after becoming

adult. Table III. shows the 15 matings in which, in addition to the bisexual progeny, each female also gave from one to seven parthenogenetic offspring. Table IV. gives the sources of the male and female progenitors of the parthenogenetic individuals over a period of years in various places in nature. Table V. indicates the further breeding of the partheno-produced [1] individuals of Table II. in matings with males. This table also includes two matings, 208 and 216, in which there were also partheno-produced offspring.

Explanation of Table II.—The second column gives the sources of the females, respectively. Those of the first 31 items had no parthenogenesis in their recorded ancestry, and are referred to Table IV. where their lines may be traced to the various places in Louisiana and Texas, where their progenitors were collected. The females of the remaining 52 items, 32–83, were the daughters, or the descendants through only one, or a few parthenogenetic, or bisexual generations of these first parthenogenetic individuals of items 1–31.

The symbols, in parentheses, in the third column indicate the factors for color patterns of the males to which the 33 females were exposed. This column is blank in those items where the females were not so exposed.

The fourth column, after the X's, shows the symbols representing the factors for the color patterns of the females. The figures, in parentheses, before these, when there was more than one, show that two or three sisters of the same genetic composition were used.

The next groups of symbols and figures represent the factors for the color patterns and the numbers of the progeny. The number of males is at the left, and the number of females at the right of the hyphen, invariably; a number after a second hyphen indicates those the sex of which was not determined, items 31, 37, 51 and 67, Table II. The last numbers, in parentheses, indicate the items, or matings in Tables II. and V. where the results of the further breeding of the progeny are shown.

Elaboration of the Use of Table II.—Item 1: Table IV. is

[1] This composite word was suggested by W. R. B. Robertson.

where the ancestry of this female, which had no recorded parthenogenesis in her line, may be traced to individuals secured, over a period of several years, at several places in Texas and Louisiana. J/Sm represents the factors for the dominant color patterns of the male to which the female, B/Cof, next after the X, was exposed. The next two groups show the progeny, two females each of the segregate patterns, B/B and Cof/Cof, respectively, and exclusively of the female of the pair.

Item 42: Bis. 204–6 indicates that these females, KΘ/S, had descended through one bisexual generation, mating 204, Table V., from the parthenogenetic female, I/S, item 6, Table II. The four groups of symbols and figures show the non-crossover and crossover progeny, all females. The final figures 234–237, in parentheses, are the numbers of the matings, Table V., which give the results of the mating with males of four of these parthenogenetic progeny.

Item 55: The female parent, B/H, had descended through one, (1), bisexual generation, not noted in these data, from mating 205, Table V., the female of which, in turn, had been one of the parthenogenetic progeny of item 7, Table II. Two of these progeny, H/H, later produced 13 females, all like themselves, item 60. Dir. 55, item 60, means that the two females came directly from the parthenogenetic progeny of item 55.

Item 75: The female parent, LΘ/M, was descended from three parthenogenetic progenitresses as follows: (*a*) A Cof/Cof female of the parthenogenetic progeny of item 4, Table II., was mated, 202, Table V. An individual from this mating was, in turn, mated, and so on for four generations, five in all, when, from this fifth bisexual generation, the LΘ/M female was taken. Bis. (4) 202–4 = four bisexual generations to mating 202, five bisexual generations in all, to parthenogenetic item 4. (*b*) Another parthenogenetic progenitress was from item 37, bred bisexually in mating 208, and then another bisexual generation, not noted in these data, to item 75. (c) The line of descent then extends from item 37, through one biparental generation, 203, to the parthenogenetic female, item 6, Table II. It is to be observed that this female, item 75, had none of the color characteristics of her three respective parthenogenetic progenitresses, items 4, 37

TABLE II.

SHOWING PARTHENOGENESIS IN *Paratettix texanus*.

Items.	Source.	Males.	Females.	Offspring.
1	Table IV	(J/Sm)	× B/Cof	B/B 0–2, Cof/Cof 0–2
2	Table IV	(Cof/KΘ)	× B/P	B/B 0–2, P/P 0–1
3	Table IV	(KΘ/P)	× B/Cof	B/B 0–1, Cof/Cof 0–1 (200)
4	Table IV	(B/KΘ)	× Cof/S	Cof/Cof 0–5, S/S 0–3 (201, 202)
5	Table IV	(B/I)	× J/N_2	J/J 0–3, N_2/N_2 0–3
6	Table IV	(Jof/K)	× I/S	I/I 0–2, S/S 0–1 (203, 204)
7	Table IV	(CofΘ/K)	× E/H	E/E 0–7, H/H 0–5 (205)
8	Table IV	(JΘ/K)	× J/P	J/J 0–2, P/P 0–1 (206)
9	Table IV	(C/EΘ)	× B/Jof	B/B 1–5, Jof/Jof 0–8
10	Table IV	(+/KΘ)	× C/N_1	C/C 0–2, N_1/N_1 0–3 (207)
11	Table IV	(+/BΘ)	× C/Jof	C/C 0–1, Jof/Jof 0–1
12	Table IV	(L/Sm)	× Hm/MΘ	Hm/Hm 0–3, HmΘ/HmΘ 0–4, M/M 0–3, MΘ/MΘ 0–5 (209, 210, 211, 212, 33, 34, 247)
13	Table IV	(K/S)	× +/J	J/J 0–1 (213)
14	Table IV	(CofΘ/E)	× Hm/Hm	Hm/Hm 0–2
15	Table IV	(N/S)	× BΘ/Hm	B/B 0–1, BΘ/BΘ 0–1, Hm/Hm 0–2, HmΘ/HmΘ 0–1
16	Table IV	(EΘ/K)	× +/B	+/+ 0–2, B/B 0–4
17	Table IV	(E/L)	× F/HΘ	F/F 0–1, H/H 0–2, HΘ/HΘ 0–6 (249)
18	Table IV		Cof/S	Cof/Cof 0–3, S/S 0–2
19	Table IV		(3) K/K	K/K 0–13
20	Table IV		(2) K/M	K/K 0–3, M/M 0–3 (233, 76)
21	Table IV		(2) C/K(Φ)	C/C 0–2, K/K 0–2, CΦ/CΦ 0–1, KΦ/KΦ 0–1 (227)
22	Table IV		(2) Jsf/Jsf	Jsf/Jsf 0–5 (35)
23	Table IV		KΘ/P	KΘ/KΘ 0–1, P/P 0–2
24	Table IV		HΘ/S_1	HΘ/HΘ 0–2
25	Table IV		(2) BΘ/H	H/H 0–4 (223, 47)
26	Table IV		(2) K/PΘ	K/K 0–3, KΘ/KΘ 0–2, P/P 0–2, PΘ/PΘ 0–4 (225, 238)
27	Table IV		(2) CofΘ/S	Cof/Cof 0–2, CofΘ/CofΘ 0–5, S/S 0–3, SΘ/SΘ 0–2.
28	Table IV		EΘ/S	EΘ/EΘ 0–1, (231)
29	Table IV		(2) Jsf/Ksf	Ksf/Ksf 0–4, Jsf/Jsf 0–1 (239, 240)
30	Table IV	(+/+)	× Hm/K	K/K 0–7, Hm/Hm 0–1
31	Table IV		H/F	H/H 0–1–1, F/F 0–1
32	Bis. 205–7	(CofΘ/E)	× H/K	H/H 0–2, K/K 0–2
33	Dir. 12	(Cof/S)	× HmΘ/HmΘ	HmΘ/HmΘ 0–6 (214, 215)
34	Dir. 12	(K/Sm)	× HmΘ/HmΘ	HmΘ/HmΘ 0–12 (216)

TABLE II. (*Continued*).

Items.	Source.	Males.	Females.	Offspring.
35	Dir. 22		Jsf/Jsf	Jsf/Jsf 0–2
36	Dir. 35	(B/KΘ)	× Jsf/Jsf	Jsf/Jsf 0–1 (217)
37	Bis. 203–6		Cof/I	Cof/Cof 0–7–1, I/I 0–9 (208, 38, 39) (Later mated to ♂ EΘ/K and produced Cof/K 0–1)
38	Dir. 37		Cof/Cof	Cof/Cof 0–3 (Same ♀ later mated 243, Table V)
39	Dir. 37		Cof/Cof	Cof/Cof 0–3
40	Bis (1) 208–37 Bis. (4) 202–4	(KΘ/L)	× B/E	B/B 0–2, E/E 0–3, (220, 221)
41	Bis. 204–6		(2) CofΘ/S	Cof/Cof 0–8, CofΘ/CofΘ 0–22, S/S 0–13, SΘ/SΘ 0–13 CofΘ/S(Θ ?) 0–1, Cof/CofΘ 0–1 (229, 230, 244, 245).
42	Bis. 204–6		(2) KΘ/S	K/K 0–11, KΘ/KΘ 0–11, S/S 0–10, SΘ/SΘ 0–17, (234, 235, 236, 237)
43	Bis.(1)229–41 Bis.(1)235–42		EΘ/S	E/E 0–3, EΘ/EΘ 0–1, SΘ/SΘ 0–1
44	Bis.(1)229–41 Bis(4)202–4 Bis.(1)235–42	(B/JofΘ)	× Cof/K	Cof/Cof 0–1, K/K 0–3 (218, 219)
45	Bis.212–12 Bis.(3)204–6	(SΘ/S_1)	× Hm/K	K/K 0–1
46	Bis.215–33	(+/+)	× Cof/HmΘ	Cof/Cof 0–2, CofΘ/CofΘ 0–1, HmΘ/HmΘ 0–2 (222)
47	Dir. 25	(CofΘ/K)	× H/H	H/H 0–3
48	Bis.(5)202–4	(F/J)	× EΘ/Ksf	KΘsf/KΘsf 0–1
49	Bis.(2)208–37 Bis.(5)202–4 Bis(2)235–42	(Cof/KΘ)	× Jsf/Psf	Jsf/Jsf 0–8, Psf/Psf 0–10
50	Bis.(5)202–4 Bis.(2)235–42 Bis.(2)208–37	(H/S_1)	× KΘsf/Psf	Ksf/Ksf 0–1, KΘsf/KΘsf 0–1, PΘsf/PΘsf 0–1
51	Bis.(1)205–7		B/K	B/B 0–3–1, K/K 0–4 (224)
52	Bis.(2)226–76 Bis.(3)211–12 Bis.(4)208–37 Bis.(2)224–51 Bis.(6)202–4 Bis.(4)235–42	(I/S_1Θ)	× B/K	B/B 0–3, K/K 0–5
53	Many, La., 1925 Bis.(2)233–20 Bis.(2)235–42		(2) +/KΘ	+Θ/+Θ 0–1

TABLE II. (*Continued*).

Items.	Source.	Males.	Females.	Offspring.
54	Bis. 205–7		(2) B/H	H/H 0–1
55	Bis.(1)205–7		B/H	B/B 0–4, H/H 0–7 (60)
56	Bis.(1)205–7		B/H	H/H 0–1
57	Bis.(1)205–7		B/H	B/B 0–2, H/H 0–4
58	Bis.(1)205–7		B/K	K/K 0–1
59	Bis.(1)205–7		B/K	B/B 0–2
60	Dir. 55		(2) H/H	H/H 0–13
61	Bis. 205–7		(2) H/K	H/H 0–15, K/K 0–4 (246)
62	Bis.(1)205–7		H/K	H/H 0–1, K/K 0–1
63	Bis.(1)205–7		H/K	H/H 0–2, K/K 0–2
64	Bis.(1)205–7		H/K	H/H 0–5 (232, 248)
65	Bis.(5)202–4		(2) Cof/EΘ	CofΘ/CofΘ 0–1
66	Bis.(1)233–20 Bis.(1)236–42		(2) CofΘ/E	Cof/Cof 0–1, E/E 0–1
67	Bis.202–4		Cof/K	Cof/Cof 0–0–4, K/K 0–4 (228)
68	Bis.204–6		(2) Cof/S	Cof/Cof 0–5, S/S 0–5
69	Bis.(1)203–6 Bis.228–67		E/K	E/E 0–3, K/K 0–3
70	Bis.(3)202–4 Bis.208–37		I/KΘ	I/I 0–1
71	Bis.(1)209–12		(2) H/KΘ	H/H 0–1, HΘ/HΘ 0–2, K/K 0–1, KΘ/KΘ 0–2
72	Bis.(1)208–37 Bis.(3)202–4		(2) I/K	K/K 0–1
73	Bis. 204–6		(2) K/S	K/K 0–8, S/S 0–10
74	Bis. 204–6		(2) K/S	K/K 0–1, S/S 0–3
75	Bis.(4)202–4 Bis.(1)208–37		LΘ/M	L/L 0–6, LΘ/LΘ 0–4, M/M 0–2, MΘ/MΘ 0–2
76	Dir. 20		M/M	M/M 1–1 (226, 226a)
77	Many, La., 1925 Bis. 222–46		(2) HmΘ/L	LΘ/LΘ 0–1 (78)
78	Dir. 77		LΘ/LΘ	LΘ/LΘ 0–24 (241, 242)
79	Bis.(2)204–6	(EΘ/M)	× Cof/K	K/K 0–1, Cof/Cof 0–1
80	Bis. 213–13		(2) Jsf/KΘsf	Jsf/Jsf 0–3, JΘsf/JΘsf 0–1, Ksf/Ksf 0–1
81	San. Ant., 1926 Bis.(3)213–13 Bis.(4)208–37	(+/+)	× EΘ/L	E/E 0–2, EΘ/EΘ 0–1, L/L 0–2, LΘ/LΘ 0–2
82	Sugarland, 1926 Bis(8)202–4 Bis.(4)213–13		(2) + Φ/PsfΘ	+ ΘΦ/+ ΘΦ 0–3, + Θsf/+ Θsf 0–1, + Φ/+ Φ 0–1, Psf/Psf 0–2, PΘsf/PΘsf 0–1
83	Sugarland, 1926 Bis.(8)202–4 Bis.(4)213–13		B/PΘsf	BΘ/BΘ 0–1, Psf/Psf 0–1, PΘ/PΘ 0–1

and 6, having lost them in the several bisexual generations (on record, but not essential to this inquiry) through which her ancestry had passed.

In a few cases the ancestries of parthenogenetic females trace back, through biparental breeding, to two, item 40, et al., three, items 44, 49, 50, or six, item 52, different parthenogenetic progenitresses.

Explanation and Illustration of the Use of Table III.—These are simple matings with the progeny showing preponderantly participation of the males in the parentage. However, one to seven individuals of each mating did not show any of the color characters of the male members of the matings, and all such were females. Since the dominant characters of the males, according to all experience, were due to show in any possible biparental progeny, these aberrant individuals are thought to have developed from unfertilized eggs.

The sources are to be read in the same way as in Table II. The ancestry of the females, only, is traced. Example, mating 101: The regions in nature from which the progenitors of the female B/S_1 came may be noted in Table IV., item 101. She had no parthenogenesis in her recorded progenitorship. Example, mating 110: The ancestry of the female parent, B/K, goes back to a progenitor, each, from Many, La., 1925, and Sugarland, Texas, 1926 (Neither of these is shown in Table IV.). Her line also goes back through six bisexual generations, not included in these data, through mating 202, Table V., to the parthenogenetic female of item 4, Table II., indicated by the Bis.(6)202–4. The Bis. (3) 235–42 means that the ancestry of this female, B/K, also goes back through three bisexual, Bis., generations, not included in the tables, to mating 235, Table V., to the parthenogenetic female of item 42, Table II.

Explanation and Illustration of the Use of Table IV.—In this table are denoted the males and females secured at various places, over a period of years, which constituted the progenitorship from nature of the 47 females, items 1–31, Tables II. and IV., and matings 100–106, Tables III. and IV., which first gave offspring parthenogenetically. The lines of ancestry of all the other 68 parthenogenetic females, Table II, and the other eight of Table III., in turn, extend back to some of these.

TABLE III.

MATINGS THAT GAVE SOME PARTHENOGENETIC INDIVIDUALS.

Mating Number.	Source.	Parents.	Offspring.
100	Table IV	P/P × +/KΘ	+/P 7–14, +Θ/P 6–12, K/P 9–12, KΘ/P 5–13, +/+ 0–2, K/K 0–1, KΘ/KΘ 0–1
101	Table IV	F/HΘ × B/S_1	B/F 1–2, B/FΘ 3–1, B/H 1–1, B/HΘ 0–1, F/S_1 2–5, FΘ/S_1 0–1, H/S_1 0–3, HΘ/S_1 2–0, B/B 0–2, S_1/S_1 0–5
102	Table IV	J/K × Cof/SmΘ	Cof/J 4–6, CofΘ/J 2–2, Cof/K 2–1, CofΘ/K 3–1, J/Sm 3–3, J/SmΘ 2–2, K/Sm 3–5, K/SmΘ 1–2, Sm/Sm 0–1
103	Table IV	CofΘ/Sm × J/P	CofΘ/J 1–1, CofΘ/P 1–0, J/Sm 4–1, J/SmΘ 0–1, P/Sm 0–2, P/SmΘ 0–1, J/J 0–1
104	Table IV	E/K × B/I	B/E 1–2, B/K 1–3, E/I 3–1, I/K 3–1, B/B 0–1
105	Table IV	Cof/K × B/JΘ	B/K 8–12, BΘ/K 5–7, J/K 4–6, J/K 2–5, JΘ/K 5–5, JΘ/K 5–3, B/Cof 11–9, BΘ/Cof 7–7, Cof/J 6–4, Cof/JΘ 12–8, BΘ/BΘ 0–1
106	Table IV	P/S × CΘ/K	C/P 0–1, KΘ/S 0–1, CΘ/S 0–1, C/S 1–0, CΘ/CΘ 0–1
107	Bis.(1)208–37 Bis.(2)202–4	B/M × IΘ/K	B/I 5–6, B/IΘ 4–4, B/K 3–2, B/KΘ 3–2, I/M 4–3, IΘ/M 3–6, K/M 4–5, KΘ/M 4–3, K/K 0–1
108	Many, La., 1925 Bis.(1)233–20	Cof/KΘ × +/M	+/Cof 4–1, +/K 1–0, +/KΘ 2–2, Cof/M 3–4, CofΘ/M 0–1, K/M 0–1, KΘ/M 3–2, M/M 0–1
109	Bis.(5)202–4 Bis.(2)208–37 Bis.(2)235–42	I/LΘ × Cof/Sm	Cof/I 9–1, Cof/IΘ 1–1, Cof/L 2–1, Cof/LΘ 4–3, I/Sm 3–2, IΘ/Sm 1–3, L/Sm 0–2, LΘ/Sm 4–0, Cof/Cof 0–1
110	Many, La., 1925 Sugarland, 1926 Bis.(6)202–4 Bis.(3)235–42	C/P × B/K	B/C 1–0, B/P 0–3, C/K 3–0, K/P 0–2, K/K 0–1
111	Bis.(7)202–4	D/S_1Θ × H/K	D/H 1–5, DΘ/H 0–2, D/K 1–3, H/S_1 3–8, H/S_1Θ 1–2, K/S_1 1–3, K/S_1Θ 4–2, H/H 0–1
112	Bis.(3)210–12	BΘ/Sm × +/Jof	+/BΘ 0–1, +/Sm 1–0, BΘ/Jof 1–0, Jof/Sm 1–0, +/+ 0–1, Jof/Jof 0–3
113	Bis.(4)209–12 Bis.(7)202–4 Bis.(5)208–37	BΘ/I × Jof/L	B/Jof 3–9, BΘ/Jof 15–7, B/L 7–4, BΘ/L 9–6, I/Jof 10–9, IΘ/Jof 9–2, I/L 12–8, IΘ/L 6–3, Jof/Jof 0–1
114	Bis.(2)202–4	B/B × H/SΘ	B/H 11–7, B/HΘ 7–7, B/S 10–7, B/SΘ 18–7, H/H 0–1

The abbreviations, in the first column, are as follows: B.R. '10, Baton Rouge, La., 1910; Many, La., 1909, 1911, 1914; Mac. '17, Mackay, Texas, near Wharton, 1917; Bea. '17, '18, Beaumont, Texas, 1917, 1918; C.S. '21, College Station, Texas, 1921; Sug. '13, '21, '22, Sugarland, Texas, 1913, 1921, 1922; Hou. '08, '11, etc., Houston, Texas, 1908, 1911, 1913, 1914, 1916, 1918, 1921, 1922; Aus. '21, '22, '23, Austin, Texas, 1921, 1922, 1923; S.A. '11, '24, San Antonio, Texas, 1911, 1924. Tables II. and III., at the bottom reading to the right, refers to the items 1–31, Table II and matings 100–106, Table III. The column of small letters above each of these indicates the number of ancestors, male or female, or both, from each place and date. Items 15, 25, in the thirteenth column, 18, 27, 28, in the sixteenth, and 23, 26, 29 and mating 103, in the twenty-third column, had the same progenitorship, respectively.

Reading from left to right, the same letters, after a place and date, denote the same individuals. Examples: The small letters, a-d, repeated 29 times, after B.R. '10, means that four individuals, males or females, or both, named a, b, c and d, respectively, collected at Baton Rouge, La., in 1910, were ancestors from nature of all, except those of the last three columns, items 2, 10 and 31. Reading to the right of Hou. '14, there were 13 individuals, a, b, c, d, e, f, g, h, i, j, k, l and m (a-m) which were in the line of descent of all except the last three, items 2, 10 and 31. Reading up from item 22, there were 71 progenitors of this female from nature; two, a and b, from S.A. '11; ten, a-d, k, l, o, p, w and x, from Aus. '22; seven, a-g, from Aus. '21; one, c, from Hou. '22; one, a, each from Hou. '21 and Hou. '18 and so on up to the top where four, a-d, were from B.R. '10. From Aus. '21, item 6 had c, d, e, f and g (c-g); item 104 had e, f, g (e-g); item 1 and mating 106 had the same four, c, d, e, f (c-f); item 3 had two, e and f, of these; all others, except items 2, 10 and 31 had these four, c-f, and, in addition, a, b and g (a-g) in their lines. The largest number of progenitors, 26 in all, was from Austin, 1922. Items 1, 4, 5, 10, and 31, are clear of these; one, y, contributed to the progenitorship of item 2, and the largest contribution, a-g, i-p, s-v and z, 20 in all, was to the female of mating 105.

Item 2 had one progenitor from Sug. '22, 2 from Hou. '22 and

TABLE IV.

WHERE PROGENITORS OF PARTHENOGENETIC INDIVIDUALS WERE COLLECTED (EXPLANATION IN TEXT).

| | | |

one from Aus. '22. The parent in item 10 came directly from a pair from S.A. '24, and 31 from a pair from New Braunfels, Texas, 1926.

This is a most intricate and, perhaps, formidable table into which many pages of writing have been condensed, but, with the aid of the references in Tables II., III. and V., one may trace the progenitorship of every parthenogenetic individual, either directly, or through one or more parthenogenetic lines, to nature where and when it became possible to begin the records.

Explanation and Example of the Use of Table V.—The second column shows the sources, in Table II., of the parthenogenetically derived females of these matings. The matings represented in this table constitute tests of the homozygosity of these females, and of the one male, matings 226, 226a. Mating 215, Table V., gave one offspring without the · for which the female parent was supposed to be homozygous. One individual, the female of mating 229, proved to be heterozygous for a factor. These cases will be reviewed in the discussion section.

Example 204: The female S/S was of the parthenogenetic progeny of item 6, Table II. The figures 41, 42, 68, 73 and 74 indicate that females from these bisexual progeny may be found to have repeated the parthenogenesis of their grandmother in the respective items, 41, 42, etc., Table II. The formula (2)79, means that some of these progeny were bred bisexually for two generations (not included in these tables) and then one of the females reproduced parthenogenetically, item 79, Table II. The last figures, (3)45 show that some of these progeny were bred three bisexual generations (not shown here), probably being mated with unrelated stocks, and then one of the females produced parthenogenetically, item 45, Table II.

Examples, matings 209–212: The females were all of the parthenogenetic progeny of item 12, Table II. The figures, in parentheses, at the right, show that some of these progeny were bred, one in 209, through one, (1) 71, bisexual generation and then two of the females used in parthenogenesis, item 71, Table II.; another one on 209, through four, (4) 113, bisexual generations to mating 113, Table III.; the one in 210, through three, (3) 112, bisexual generations to mating 112, Table III; the one in 211

TABLE V.

TESTING PARTHENOGENETICALLY PRODUCED INDIVIDUALS.

No.	Source of Female.	Parents.	Offspring.
200	3	JΘ/K × B/B	B/JΘ 1–0
201	4	K/PΘ × Cof/Cof	Cof/KΘ 1–0, Cof/PΘ 0–1
202	4	EΘ/K × Cof/Cof	Cof/E 0–1, Cof/EΘ 3–2, Cof/K 6–4, Cof/KΘ 2–1 67, (2) 115, (3) 70, 72, (4) 40, 44, 75, 107, (5) 48, 49, 50, 65, 109, (6) 52, 110, (7) 111, 113, (8) 82, 83, 114
203	6	Cof/E × I/I	E/I 4–4, Cof/I 7–8 37, (1) 69
204	6	CofΘ/K × S/S	Cof/S 2–2, CofΘ/S 7–4, K/S 5–8, KΘ/S 1–2 41, 42, 68, 73, 74, (2) 79, (3) 45
205	7	B/K × H/H	B/H 4–3, H/K 5–6, 32, 54, 61, (1) 51, 55, 56, 57, 58, 59, 62, 63, 64, (7) 114.
206	8	I/K × J/J	I/J 2–1
207	10	K/SΘ × C/C	C/K 0–2
208	37	K/LΘ × I/I	I/K 18–13, I/KΘ 6–5, I/L 6–3, I/LΘ 17–9, I/I 0–1, 70, (1) 40, 72, 75, 107, (2) 49, 50, 109, (3) 52, (4) 81, (5) 113
209	12	KΘ/N × M/M	NΘ/M 1–4, K/M 2–0, KΘ/M 5–5, N/M 3–3, (1) 71, (4) 113
210	12	Jof/Jof × MΘ/MΘ	Jof/MΘ 1–1, (3) 112
211	12	K/LΘ × Hm/Hm	Hm/LΘ 2–0, Hm/K 0–1, Hm/KΘ 0–1, (3) 52
212	12	CofΘ/K × Hm/Hm	Hm/K 3–3, Hm/KΘ 0–1, CofΘ/Hm 1–1, (1) 45
213	13	KΘ/P × J/J	J/KΘ 3–7, J/P 3–2, J/PΘ 2–2, J/K 5–2, (1) 80, (3) 81, (4) 82, 83
214	33	B/K × HmΘ/HmΘ	B/HmΘ 3–4, HmΘ/K 5–3
215	33	Cof/K × HmΘ/HmΘ	HmΘ/K 27–14, Cof/HmΘ 24–18, Cof/Hm 1–0, (1) 46, (3) 114
216	34	E/S × HmΘ/HmΘ	E/HmΘ 2–1, HmΘ/S 2–2, HmΘ/HmΘ 0–1
217	36	KΘ/P × Jsf/Jsf	Jsf/K 4–2, Jsf/KΘ 10–8, Jsf/P 14–9, Jsf/PΘ 7–3
218	44	Cof/EΘ × K/K	Cof/K 1–0, CofΘ/K 2–0, E/K 1–0, EΘ/K 4–1
219	44	Cof/EΘ × K/K	E/K 1–0, EΘ/K 3–1
220	40	JΘ/K × B/B	B/J 1–1, B/JΘ 1–5, B/K 1–5, B/KΘ 0–1 (2) 114
221	40	J/KΘ × E/E	E/J 4–5, E/JΘ 1–1, E/KΘ 4–3
222	46	L/Sm × HmΘ/HmΘ	HmΘ/L 2–3, HmΘ/Sm 2–4 77
223	25	B/CofΘ × H/H	B/H 1–3, CofΘ/H 1–0

TABLE V. (*Continued*).

No.	Source of Female.	Parents.	Offspring.
224	51	I/KΘ × B/B	B/KΘ 0–2, B/IΘ 0–2 (2) 52
225	26	B/S × PΘ/PΘ	B/PΘ 6–10, PΘ/S 4–6
226	76	M/M × Cof/L	L/M 13–6, Cof/M 15–11 (2) 52
226a	76	M/M × B/K	B/M 15–15, K/M 23–14
227	21	CofΘ/E × K/KΦΦ	Cof/KΦ 1–0
228	67	E/I × K/K	E/K 4–3, I/K 8–3 69
229	41	K/S × Cof/CofΘ	Cof/K 2–3, Cof/S 6–6, CofΘ/K 6–3, CofΘ/S 4–5 (1) 43, 44
230	41	+/Jof × SΘ/SΘ	+/SΘ 5–1, Jof/SΘ 2–4
231	28	K/N1 × EΘ/EΘ	EΘ/K 0–1
232	64	CofΘ/K × H/H	Cof/H 0–1, CofΘ/H 2–4, H/KΘ 2–0
233	20	CofΘ/E × M/M	E/M 11–10, EΘ/M 8–2, Cof/M 9–6, CofΘ/M 12–9 (1) 66, 108, (2) 53, (5) 114
234	42	Cof/EΘ × S/S	Cof/S 3–0, CofΘ/S 1–0, E/S 1–2, EΘ/S 7–2
235	42	EΘ/L × K/K	E/K 2–3, EΘ/K 7–5, K/L 13–8, K/LΘ 1–0 (1) 43, 44, (2) 49, 50, 53, 109, (3) 110, (4) 52
236	42	CofΘ/E × K/K	E/K, +EΘ/K 7–5, Cof/K 6–3, CofΘ/K 4–2 (1) 66
237	42	B/H × SΘ/SΘ	B/SΘ 15–13, H/SΘ 10–14 (5) 114
238	26	BΘ/Hm × K/K	B/K 1–0, BΘ/K 2–1, Hm/K 1–3, HmΘ/K 1–0
239	29	K/LΘ × Ksf/Ksf	Ksf/K 0–2, Ksf/L 1–0
240	29	+/SΘ × Jsf/Jsf	+/Jsf 2–4, Jsf/S 1–0, Jsf/SΘ 6–3
241	78	C/S1 × LΘ/LΘ	C/LΘ 1–0
242	78	C/S1 × LΘ/LΘ	C/LΘ 2–1, LΘ/S1 0–2
243	37	JΘ/K × Cof/Cof	Cof/J 7–4, Cof/JΘ 15–7, Cof/K 6–8, Cof/KΘ 6–4
244	41	M/Sm × CofΘ/CofΘ	CofΘ/Sm 1–1, CofΘ/M 2–1
245	41	I/K × Cof/Cof	Cof/I 2–5, Cof/K 6–1
246	61	I/LΘ × K/K	I/K 0–1, IΘ/K 0–1, K/LΘ 0–1
247	12	Cof/K × MΘ/MΘ	K/MΘ 3–5, Cof/MΘ 6–0
248	64	CofΘ/K × H/H	CofΘ/H 1–0, H/K 1–1, H/KΘ 1–2, Cof/H 0–1
249	17	Cof/K × F/F	Cof/F 1–0

through three, (3) 52, bisexual generations to item 52, Table II., and the one in 212 through one, (1) 45, bisexual generation to item 45, Table II., respectively.

Discussion.

The comparatively sudden inception of a considerable degree of parthenogenesis in *P. texanus,* beginning in 1922, after fourteen years of perhaps exclusively bisexual reproduction, implied the recent introduction, prior to this date, of some causative factor, or factors. Then came the proposals of Peacock and Harrison (1925, 1926): (1) The generalization, deduced largely from the results of their own experiments, *that parthenogenesis was consequent upon hybridity,* and, (2) from my data, that the rather extensive parthenogenesis exhibited by *A. eurycephalus* (Nabours, 1919, 1925) was the result of hybridizing one variety of this species from Tampico, Mexico, with another from the region of Houston, Texas.

In 1921, the year preceding the onset of this period of parthenogenesis in *P. texanus,* specimens had been secured at Austin and College Station, Texas, new areas along the northeastern boundary of the range of the species (Table IV.). It was noted that all the parthenogenetic individuals, 1922–1926, had in their ancestry progenitors from these new areas, except that those of item 3, Table II. and IV., did not connect with College Station. Item 2, Table II. and IV. had one ancestor from Austin, 1922. The females of items 10 and 31 were from parents collected at San Antonio, 1924, and New Braunfels, 1926, respectively.

The arrangements of the data in the tables, especially Table IV., have been influenced by these considerations. The ancestry of all the parthenogenetic individuals may be traced to their various origins in nature. The 40 females of items 1–31, Table II., and 7 of matings 100–106, Table III., had no recorded parthenogenetic progenitresses; the other 68 females of Table II., and 8 females of Table III., in turn, were descendants of these.

An examination of Table IV. reveals a very complex ancestry for these 47 females which first displayed this characteristic, and from which all the other parthenogenous ones were descended. But it appears to have remained for the introduction of the speci-

mens from Austin, 1921, to bring in the factor, or *the complementary climaxing factor, or factors,* responsible for the significant measure of parthenogenesis which ensued.

However, possibly opposing this tentative suggestion were the two cases of parthenogenesis, items 10 and 31, Table IV., directly from San Antonio and New Braunfels (about half way between San Antonio and Austin), respectively. The female of item 2, Tables II., and IV., had few recorded progenitors, but one of them was from Austin, 1922. It is difficult to estimate the importance of the three cases of probable parthenogenesis of 1912 and 1915 (see pp. 130, 131).

The narrow confines of Shoal Creek, near Austin, is the only new area that contributed to the progenitorship of all the parthenogenetic females, except items 10 and 31, beginning in 1921. The female of 10 was from San Antonio, but taken 13 years after the two original progenitors (Table IV.). The female of item 31 was from New Braunfels, which was also a new area, near Austin. The factor, or factors responsible for parthenogenesis may even have been contributed from Houston, or Sugarland, 1922, the several previous collections from these areas having possibly simply missed the parthenogenetic strain, if such there was.

It was urgent that a strain highly parthenogenous should be hybridized with one which was not so at all, and then the causative factors recovered. A few such experiments have been attempted, the least unsatisfactory one having been conducted as follows: The male B/K, mating 205, Table V., had no parthenogenesis in his recorded ancestry. The female, H/H, was a parthenogenetic product, item 7, Table II. They gave bisexually both males and females in F_1. Eight of the nine female F_1 progeny were disposed of as follows: Four unmated, two in one cage and two in another, gave offspring, items 54, 61, Table II. (obviously one of those in 54 did not reproduce). Three were mated out; one gave no offspring, one gave bisexual (not included in the tables) and the other parthenogenetic progeny, item 32, Table II. The eighth female was mated to a brother and they gave a numerous F_2 progeny (not included in tables). From these F_2 (parthenogenetic by nonparthenogenetic?) progeny, eighteen females were separated, one each, in cages; nine gave offspring, and two

others were known to have laid a batch of eggs each which were not given opportunity to hatch, items 51, 55–59, 62–64, Table II. Some of the other seven may have laid eggs, but they did not reproduce. The quantitative results of this experiment thus appeared to constitute a superb and convincing case of parthenogenesis as a dominant Mendelian characteristic.

However, the following facts concerning the conditions of the experiment should be taken into consideration: Five of the F_2 females, the first to become adult, had been placed in their respective cages on May 23; the other 13 had been placed on June 14, twenty-two days later, that much farther on into the hot summer, and past the optimum breeding season which was March–May (See Table I.). The records show that the five placed first all gave progeny of 3, 9, 17, 17, and 20, respectively. Only three of the 13 females, placed 22 days later, gave offspring, two hatching one each, and the other, five. As noted above, at least two others laid batches of eggs. The question is still open: What would have been the result if all eighteen of these F_2 females had been placed May 23 or earlier? Or, what would have happened if all of them had been placed on June 14, or later?

All the females and one male of *A. eurycephalus* hatched from unfertilized eggs, which were tested by further breeding, proved to be homozygous for all the observable characteristics. Obviously, it could not be stated categorically that any of the untested ones were homozygous since the color patterns were dominant (Nabours, 1919, 1925). The females and one male of *P. texanus,* so far as they were tested, were also homozygous, except that one individual of item 41, Table II., had the appearance of having both the alternatives Cof and S, and another also from 41, was proved to be heterozygous for ♂, mating 229, Table V. Mating 215, Table V., contained one Cof/Hm, the only one in 84 without the ♀ for which the female parent was supposed to be homozygous. Cof and S are certainly very closely linked, if not precisely alternative; so it is unlikely that there was crossing over. The failure to breed this animal leaves the question of her actual genetic composition in obscurity. About the Cof/Cof♀ individual, there was only the question of the cause. The factor for ♀ appears to be near the end of the chromosome (Haldane, 1920).

As is shown by W. R. B. Robertson (MSS, 1929, Nabours, 1929), the homologous chromosomes of the germ and somatic cells of the parthenogenetic offspring have a tendency to lie together in pairs, often even adhering, so that a pair may appear as one, double in size. Pending further developments, it may be suggested that these aberrances were brought about through, perhaps, an adherance, in the one case, and an elimination of the ends of the chromosomes containing θ, in the others.

The inheritance results in parthenogenesis indicate that segregation and crossing over occur before the inception of the parthenogenetic processes, and that up to this stage in gametogenesis there is no essential departure from the usual procedure in bisexual reproduction (Nabours, 1925, 1929).

Dr. W. R. B. Robertson, working in our laboratory, has discovered that the numbers of chromosomes in some of the soma cells, likewise the oögonial cells, of the parthenogenetically produced females of *A. eurycephalus* and *P. texanus* appear to range from seven to fourteen, though there are actually fourteen in all of them. When the full fourteen are manifest, the members of the homologous pairs, respectively, lie together in early cell division, and not far apart, each from the other in later cell generations, in such positions as to suggest the second polar body division had been inhibited.

When only seven chromosomes appear to be present in these parthenogenetically produced individuals, *each has twice the bulk in transverse thickness* (*broadness of the equatorial plane*) *of either member of the similarly numbered pair in the cells containing seven discreet pairs.* Furthermore, in these parthenogenetically produced grouse locusts, Robertson notes that in those soma and oögonial cells *containing above seven chromosomes, there is one less of the broad chromosomes for every additional one above seven.* For example, if the *apparent* number is nine, there are five of double equatorial broadness, or bulky ones, and four smaller ones in two pairs, the homologues of which always lie together, or not far from each other, in the same manner as the similar pairs do in those cells of the parthenogenetically derived females containing the fourteen discreetely paired chromosomes. An analogous situation is found in the cells of the parthenogeneti-

called derived males which he has examined (Robertson, 1929, MSS., Nabours, 1929).

The entrance of the sperm is probably necessary to the second polocyte division in the *Tettigidae* (loc. cit.), as in other forms, another "case of a later stage of maturation being overlapped by an earlier stage of fertilization" (Wilson, 1925). Fertilization lacking, there is a resultant partial or complete inhibition of the last polocyte division which restores or retains the condition of diploidy (See Robertson's observations on the chromosomes pp. 151, 152 and in MSS., and Nabours, 1929). When the specific gene or complementary genes responsible for parthenogenesis are present, development is initiated. As already stated (pp. 131, 132), probably no female would be partheno-producing if mated with a potent male (those not carrying the specific genes probably require fertilization). Therefore such initiation of development as this might, in effect, be termed *artificial* parthenogenesis.

The kind of parthenogenesis shown by the grouse locusts can hardly be called *facultative,* and certainly not *obligatory* (See definitions, Wilson, 1925, pp. 228, 229). It might perhaps be best entitled *tychoparthenogenesis,* and preponderantly *gynogenetic.*

In a parthenogenetic line of *P. texanus,* a female exposed to a male gave parthenogenetic progeny. Seven of these were mated out; five gave biparental offspring, but the other two again gave parthenogenetic progeny in spite of the males to which they had been exposed. However, the progeny of the second parthenogenetic generation, when exposed to males, mated and gave offspring from fertilized eggs (items 12, 33, 34, Table II., and matings 209–212, 214–216, Table V.).

There have been other cases of two succeeding parthenogenetic generations while the females were exposed to males in *P. texanus* and *A. eurycephalus* (not included in the attached data). It has been thought that a female might mutate in some way which would render her incapable of mating with a male of her own species. In such a case, she should reproduce by parthenogenesis. Then, if her progeny, due to the same mutation, could not mate back to the males of the original stock, and parthenogenesis should go on till the occasional male appears (see items 226, 226a, Table

V., and mating 5027, Nabours, 1925), the "event for which we wait" (Bateson, 1922) might be achieved. However, it is likely that the cases of succeeding generations by parthenogenesis, although the females were exposed to males, were due to impotency of the males rather than to mutations in the females. Nevertheless, there may be possibilities in this direction.

The hypothesis of Peacock and Harrison that parthenogenesis is consequent upon hybridity (loc. cit.) has probably received further support from the results of the parthenogenetic breeding of *P. texanus*, as described, if it be provided in addition *that the process of hybridism may bring together specific complementary, or climaxing genes which are responsible for, or cause the development of unfertilized eggs.*

Summary and Conclusions.

1. There is indication of a genetic factor, or a group of complementary factors responsible for parthenogenesis in these grouse locusts.

2. The hypothesis of Peacock and Harrison (1925, 1926) that parthenogenesis is consequent upon hybridity is probably further supported if it be provided, as these authors did not, that it is necessary to bring together in the processes of hybridism the specific complementary, or climaxing genetic factors which may cause the development of unfertilized eggs.

3. The members of the species *Paratettix texanus* Hancock are bisexual, the fertilized eggs producing males and females in equal numbers, and parthenogenetic, the unfertilized eggs, with rare exceptions, hatching females.

4. A mated female may have part of her ova fertilized, and also produce from unfertilized ova, by parthenogenesis, an additional number of offspring which are nearly always females.

5. The segregation and crossing over of factors occur in individuals reproducing by parthenogenesis to the same extent as in those reproducing bisexually.

6. If fertilized, the egg proceeds with the second polocyte division and develops bisexually. In the absence of the fertilizing sperm the last polyocyte division does not occur, or if it does the polar body is not eliminated. If the specific complementary gene,

or genes responsible for initiating parthenogenesis be present, diploidy is retained, or restored, and development may begin, consequentially as a kind of *artificial* parthenogenesis.

7. The progeny from unfertilized eggs are usually homozygous for all the factors they carry, though rarely one proves to be heterozygous for factors.

LITERATURE CITED.

Bateson, Wm.

'22 Evolutionary Faith and Modern Doubts. Science, 55: 55–61.

Bellamy, A. W.

'17 Multiple Allelomorphism and Inheritance of Color Patterns in *Tettigidea*. Jour. Genetics, Vol. VII., No. 1, pp. 55–70.

Cuénot, L.

'09 Les Males d'abeilles proviennent—ils toujours d'oeufs parthenogenetiques. Bull. Sci. France et Belg., XLIII.

Haldane, J. B. S.

'20 Note on a case of Linkage in *Paratettix*. Jour. Genetics, Vol. X., No. 1, pp. 47–51.

Hancock, J. L.

'02 The *Tettigidæ* of North America. Chicago, 188 pp.

Harman, Mary T.

'15 Spermatogenesis in *Paratettix*. Biol. Bull., Vol. XXIX., pp. 262–277.

Nabours, Robert K.

'14 Studies of Inheritance and Evolution in Orthoptera I. *Paratettix texanus*. Jour. Genetics, Vol. III., No. 3, pp. 141–170.

'17 Studies of Inheritance and Evolution in Orthoptera II. and III.: *Paratettix texanus* and a Mutant. Jour. Genetics, Vol. VII., No. 1, pp. 1–54.

'19 Parthenogenesis and Crossing Over in the Grouse Locust *Apotettix*. Am. Nat., Vol. LIII., No. 625, pp. 131–142.

'23 A New Dominant Color Pattern and Combinations that Breed True in the Grouse Locusts. Genetica, Vol. V., Nos. 5 and 6, pp. 477–480.

'25 Studies of Inheritance and Evolution in Orthoptera. V. The Grouse Locust, *Apotettix eurycephalus* Hancock. Kan. Agri. Exp. Sta., Tech. Bull. 17: 1–231.

'27 Polyandry in the Grouse Locust, *Paratettix texanus* Hancock, with Notes on the Inheritance of Acquired Characters and Telegony. Am. Nat., Vol. LXI., pp. 531–538.

'29 The Genetics of the Tettigidæ (Grouse Locusts). Bibliographica Genetica, Vol. 5, pp. 27–101.

Nabours, Robert K., and Snyder, Bertha.

'28 Parthenogenesis and the Inheritance of Color Patterns in the Grouse Locust, *Telmatettix aztecus* Saussure. Genetics, Vol. XIII., pp. 126–132.

Newell, Wilmon.

'14 Inheritance in the Honey Bee. Science, N. S., Vol. XLI., No. 1049, pp. 218–219.

Peacock, A. D., and Harrison, J. W. Heslop.

'25 On Parthenogenesis Originating in Lepidopterous Crosses. Trans. Nat. Hist. Soc. Northumberland, Durham and New Castle-upon-Tyne, N. S., 6, Part II.

'26 Hybridity, Parthenogenesis and Segregation. Nature, 117: 378–379.

Perez.

'79 The Theory of Dzierzon. Am. Bee Jour., XV.

Robertson, W. R. B.

'15 Chromosome Studies, III. Inequalities and Deficiencies in Homologous Chromosomes: Their Bearing upon Synapsis and the Loss of Unit Characters. Jour. Morph., Vol. XXVI., pp. 109–141.

'29 Chromosome Studies, V.: The Retention of Diploidy in Partheno-produced *Tettigidæ*, including two rare males, and the Tendency of Chromosomes to Persist in Their Original Gametic Position in the Cells of These and Their Biparentally Produced Relatives (*Apotettix eurycephalus* and *Paratettix texanus*). (In manuscript.)

Whiting, P. W.

'21*a* Studies on the Parasitic Wasp, *Hadrobracon brevicornis* (Wesmael), I. Genetics of an Orange-eyed Mutation and the Production of Mosaic Males from Fertilized Eggs. BIOL. BULL., Vol. XLI., No. 1, pp. 42–54.

'21*b* Studies on the Parasitic Wasp, *Hadrobracon brevicornis* (Wesmael), II. A Lethal Factor Linked with Orange. BIOL. BULL., Vol. XLI., No. 3, pp. 153–155.

Wilson, Edmund B.

'25 The Cell in Development and Heredity. Macmillan, New York, 1232 pages.